Stromal cell contribution in homeostatic and pathogenic immune responses

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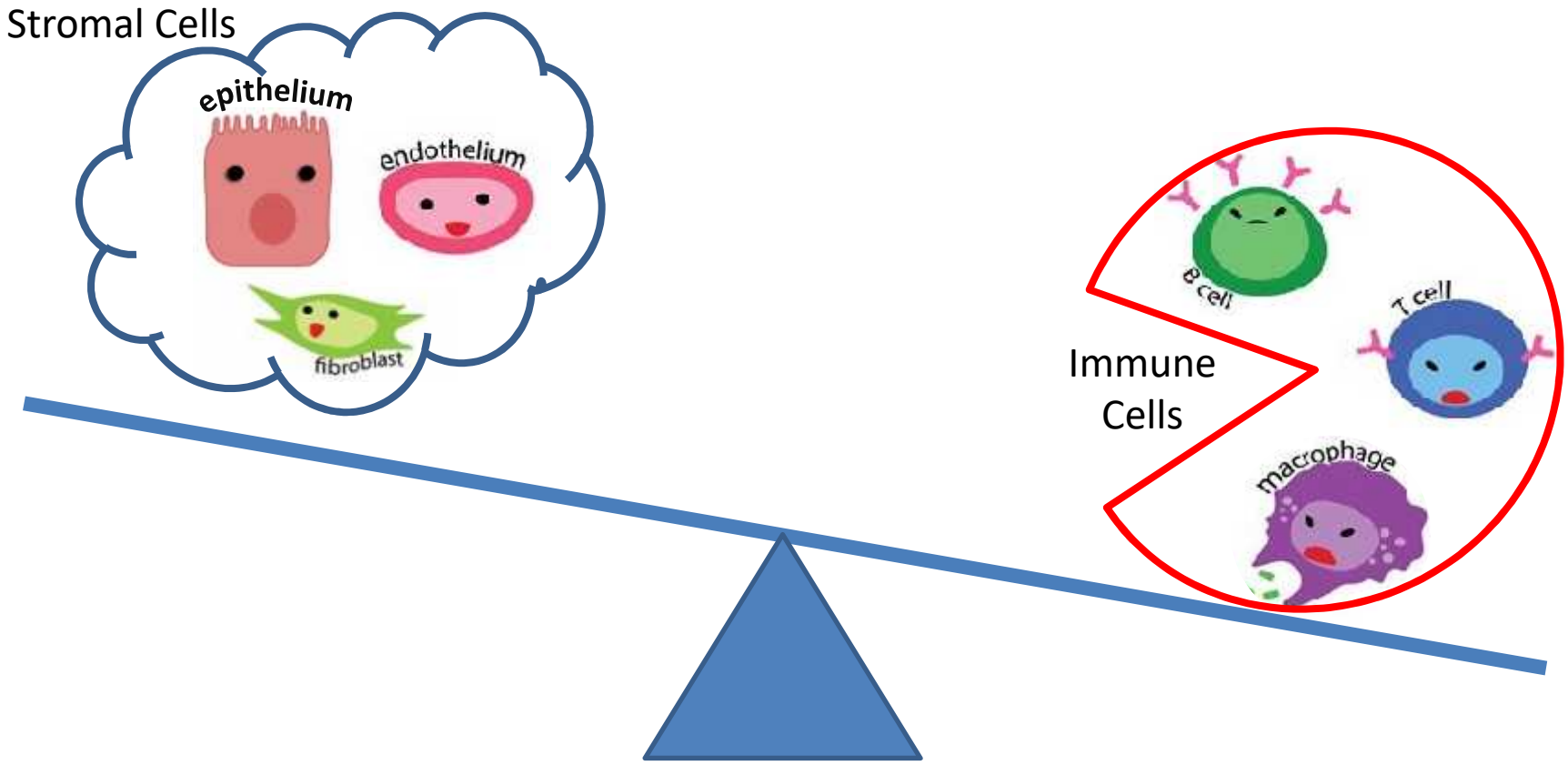
BSRC Alexander Fleming

2nd Immunology Workshop for Clinicians
1-3 November 2019 | Heraklion, Greece

Stromal cell contribution in homeostatic and pathogenic immune responses



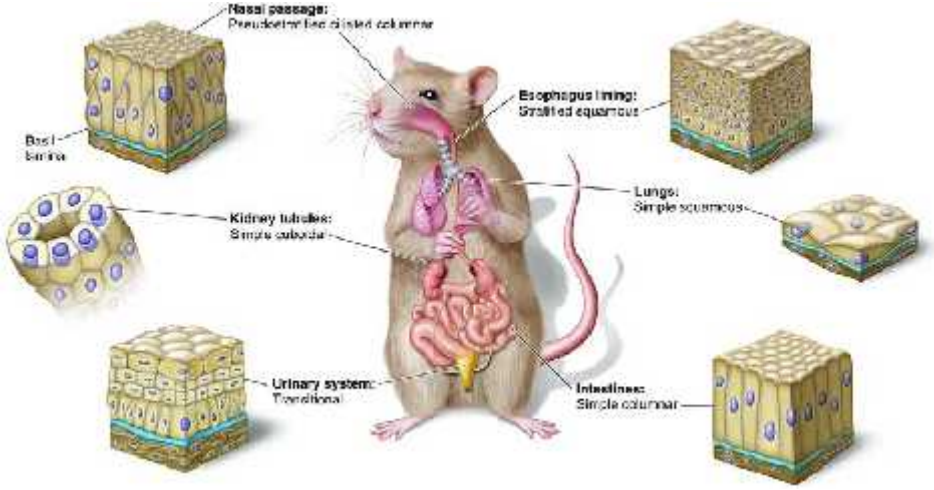
Stromal cell contribution in homeostatic and pathogenic immune responses

Outline

- Epithelium and endothelium
  Homeostatic /immune functions and the crosstalk with resident mesenchymal cells

- Connective tissue
  Mesenchymal cell: Fibroblasts
    Homeostatic/immune functions
    Fibroblasts and RA

Epithelial Tissue

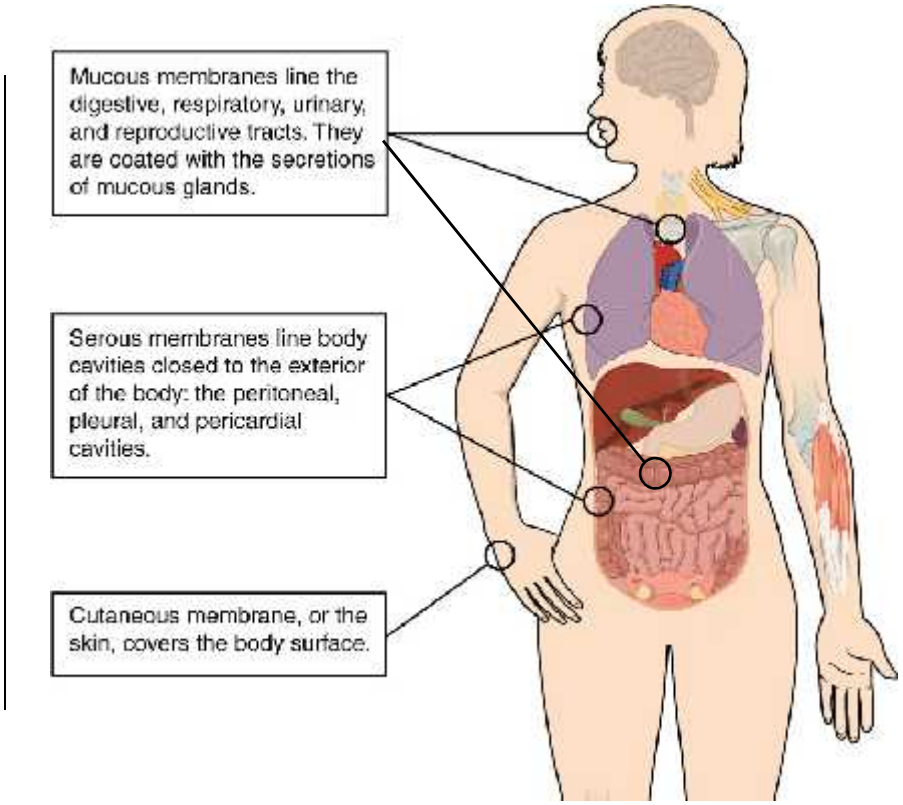


epithelium: A membranous tissue composed of one or more layers of cells that form the covering of most internal and external surfaces of the body and its organs.

avascular: Lacking blood vessels.

vascular: Containing blood vessels (Stria vascularis within ear; vascularized stratified epithelium)

Epithelial Membranes

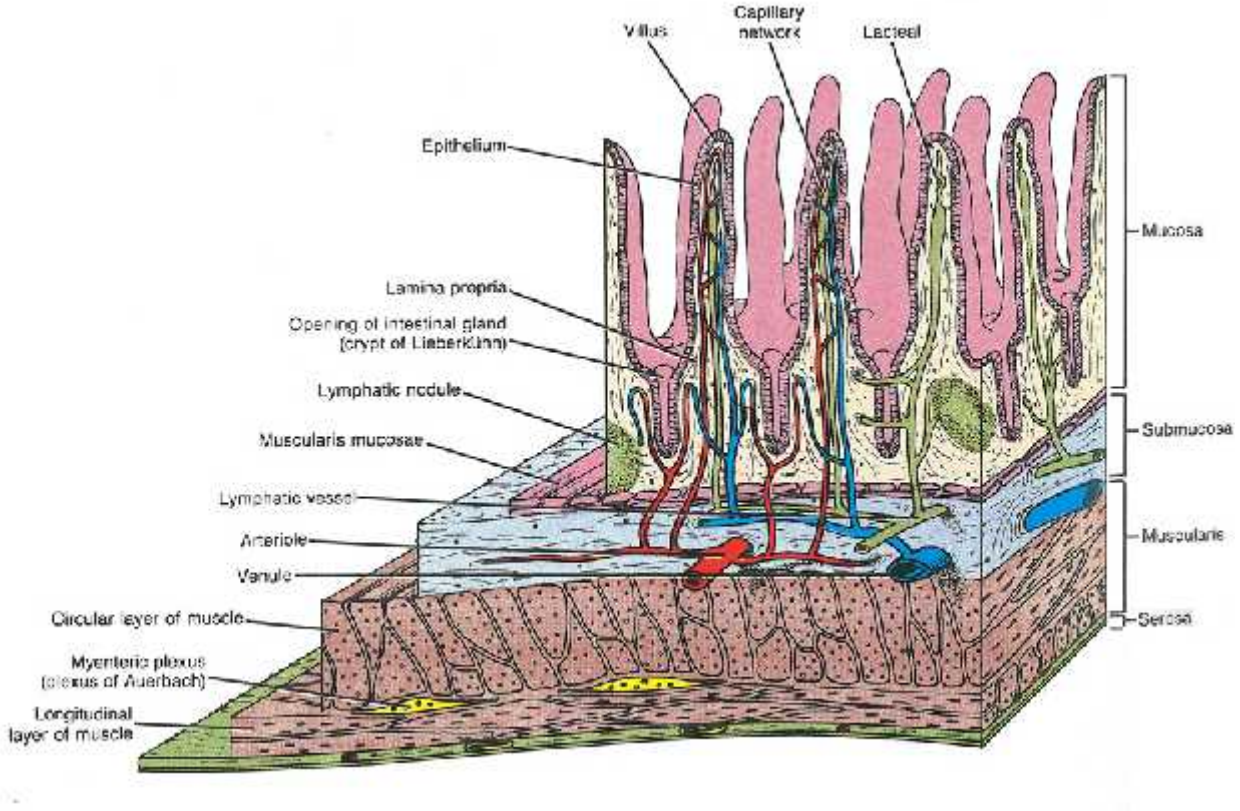


Epithelial Membranes

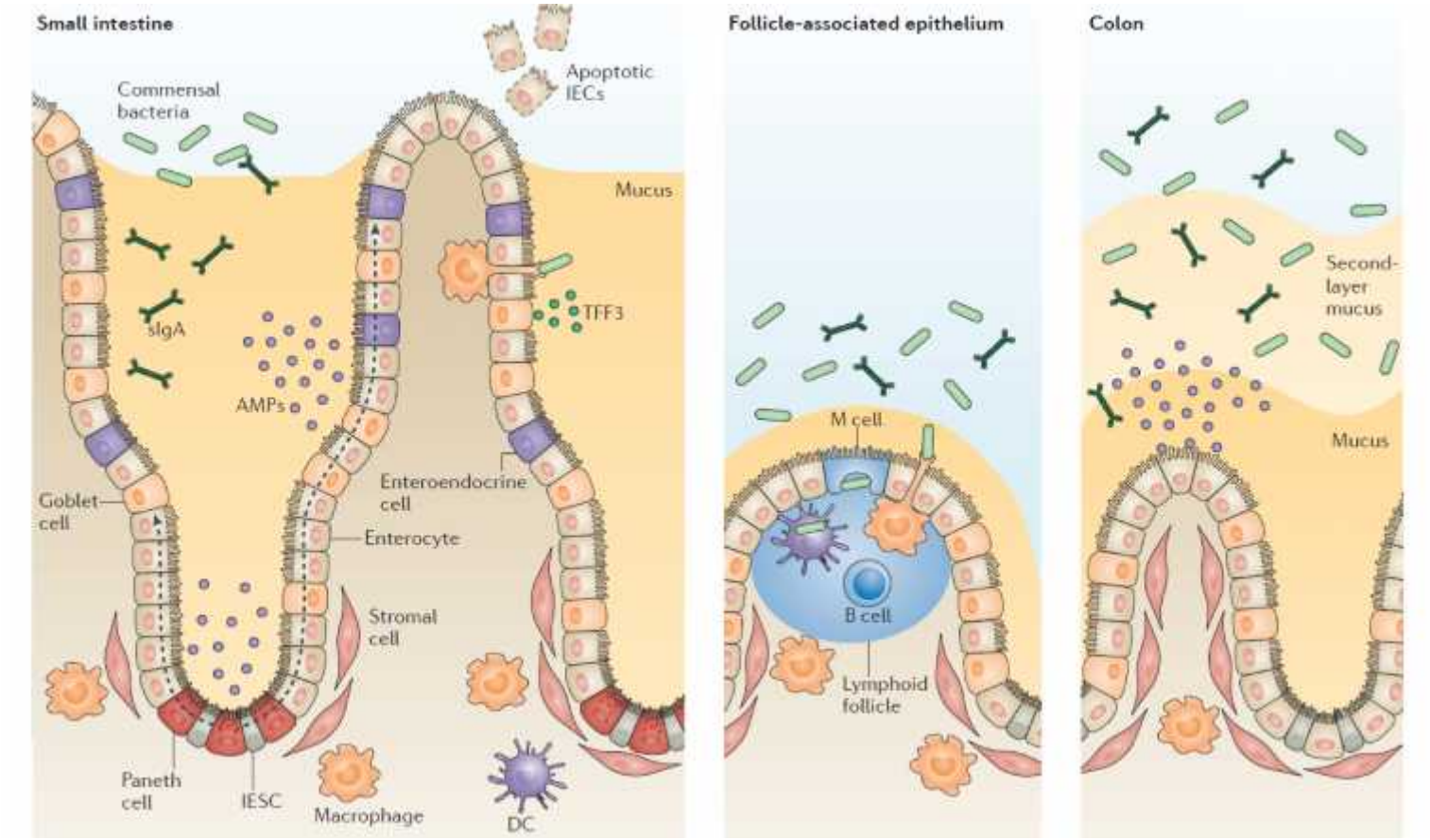
Basic Functions

1. Provide physical protection
2. Control permeability
3. Move fluids over the surface
4. Provide sensation
5. Produce specialized secretions

Intestine (mucus epithelial membrane)



Intestine (mucus epithelial membrane)



Nature Reviews Immunology 2014

Immune functions of intestinal epithelial cells

dynamic sensors of the microbial environment

Secretory goblet cells and Paneth cells
>>>>secrete mucus and antimicrobial proteins (AMPs)
>>>>facilitate the transcytosis and luminal release of secretory IgA (sIgA) further contribute to this barrier function.

Microfold cells (M cells) and goblet cells
>>>>>mediate transport of luminal antigens and live bacteria across the epithelial barrier to dendritic cells (DCs) and intestine-resident macrophages sample the lumen through transepithelial dendrites

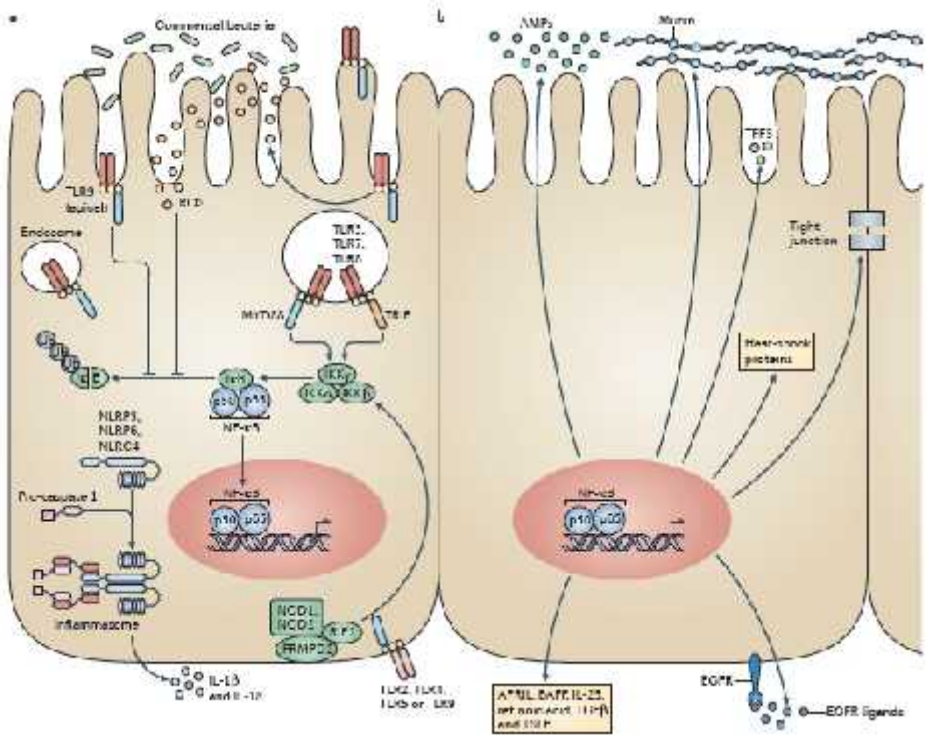
Immune functions of intestinal epithelial cells

dynamic sensors of the microbial environment

IECs express pattern-recognition receptors

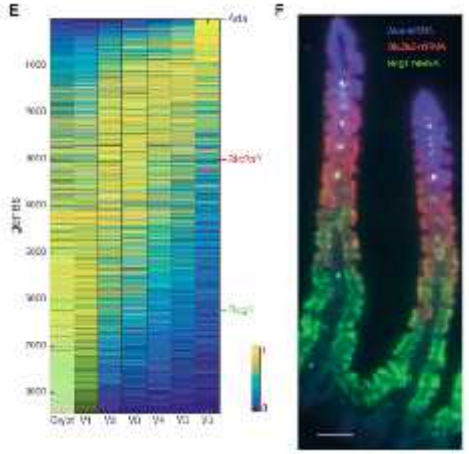
- Members of the Toll-like receptor (TLR),
- NOD-like receptor (NLR) and
- RIG-I-like receptor (RLR) families

providing distinct pathways for the recognition of microbial ligands or endogenous signals associated with homeostasis and pathogenesis



Nature Reviews Immunology 2014

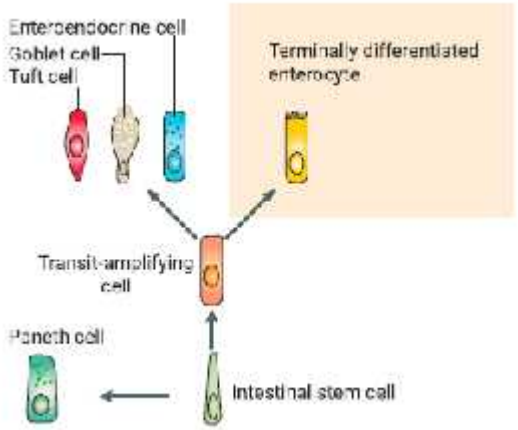
SC transcriptomics:
Unexpected spatial heterogeneity within small intestinal enterocytes



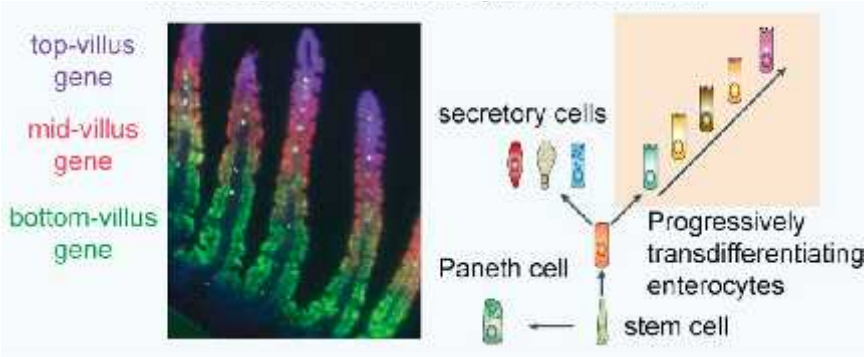
Immunomodulatory enterocytes (eg. Dominant CD73 expression)

Gatekeeper enterocytes
(complementing Paneth cell?)

Classic view of enterocyte differentiation



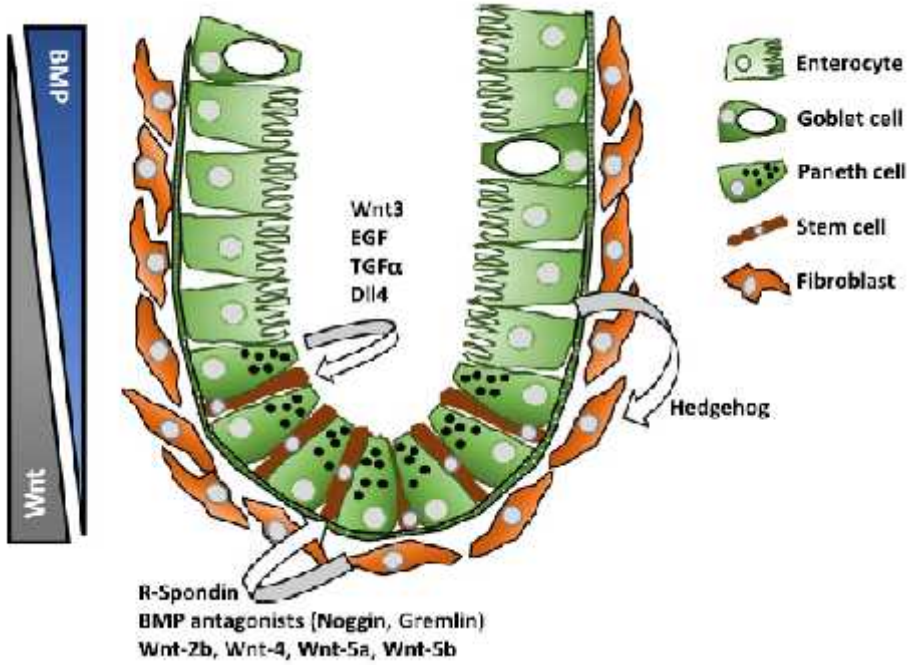
Refined view of enterocyte differentiation



Cell 2018

Intestinal mesenchymal cells in homeostasis

Intestinal mesenchymal cells as part of the crypt stem cell niche

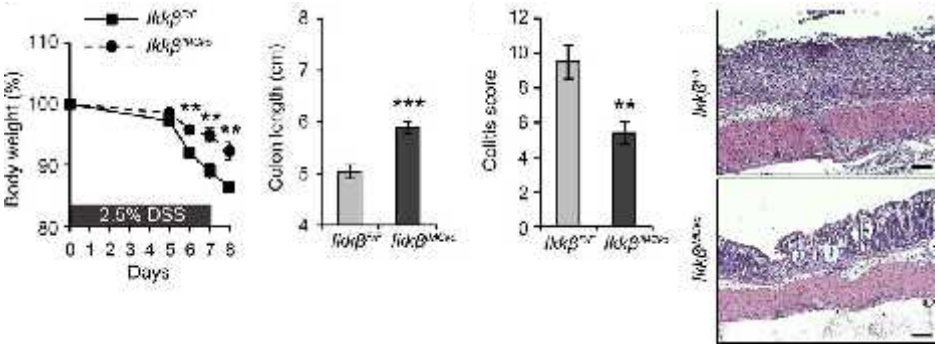


Differentiation 2016

Intestinal mesenchymal cells in inflammation

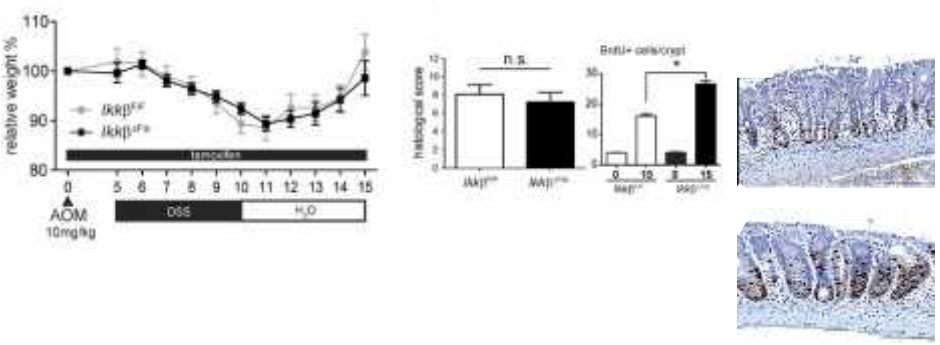
Intestinal mesenchymal cells shape the inflammatory colitogenic response in murine model of colitis

Collagen VI expressing IMCs



JEM 2015

Collagen I expressing IMCs

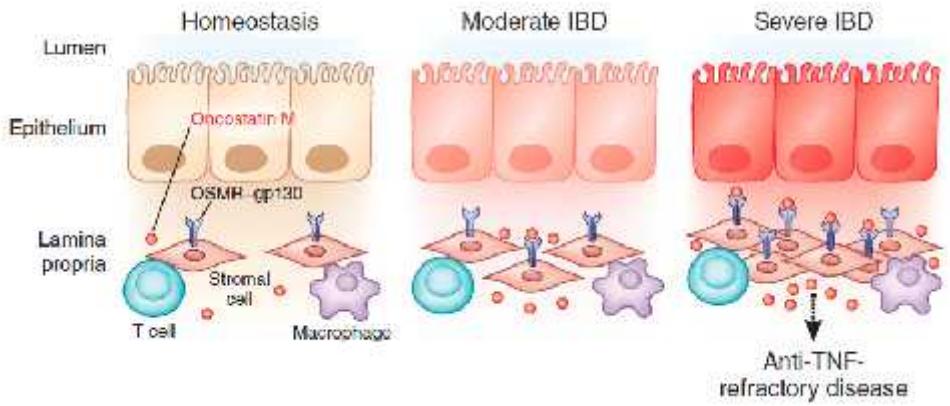


JEM 2015

Intestinal mesenchymal cells in inflammation

Intestinal mesenchymal cells as part of the inflammatory response in IBD

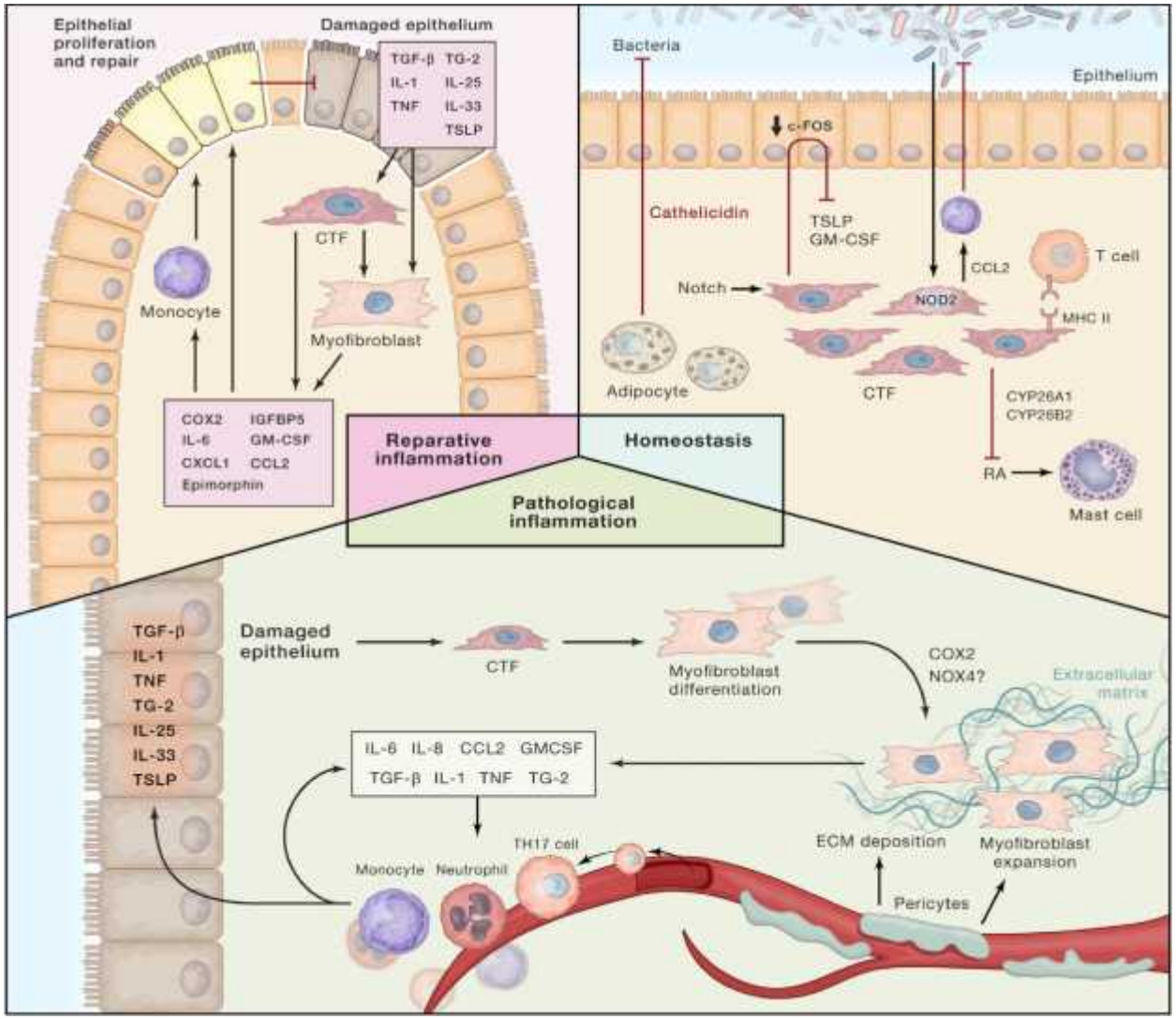
Oncostatin M: the most transcriptionally upregulated cytokine in inflamed intestinal mucosa from patients with CD and as among the most upregulated in patients with UC



Mesenchymal cells as key players in OSM-mediated inflammation in human and mouse studies

Nature Medicine 2017

Epithelial/mesenchymal barrier function overview



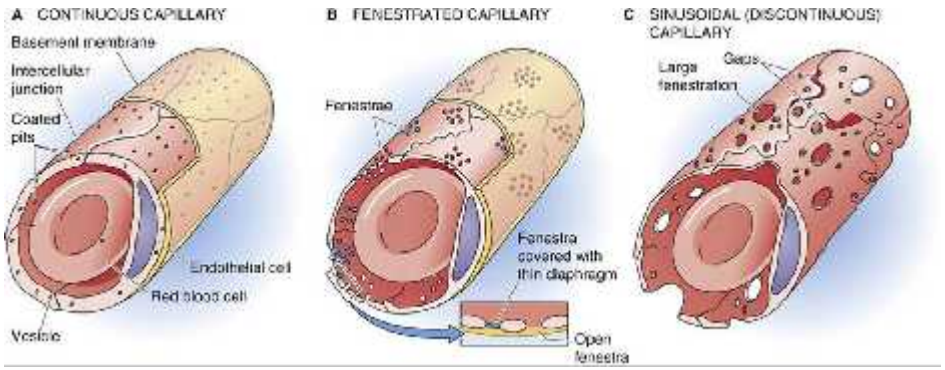
Cell 2017

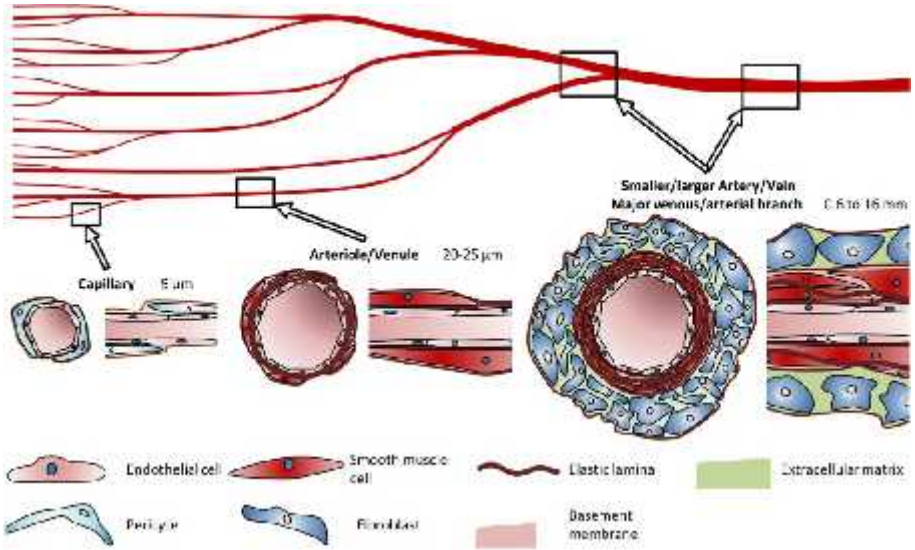
Endothelium

Most blood vessels consist of three layers:

- The outer layer (adventitia) is mostly connective tissue: collagen fibers, some elastic fibers.
- The middle layer (media) contains mix of smooth muscle and elastic fibers. This components of this layer vary the most. Elastic arteries such as the aorta contain more elastic fibers to generate stretch and recoil.
- **The inner layer of blood vessels** is called endothelium and it is composed of a simple squamous epithelium-like lining (Endothelial cells).

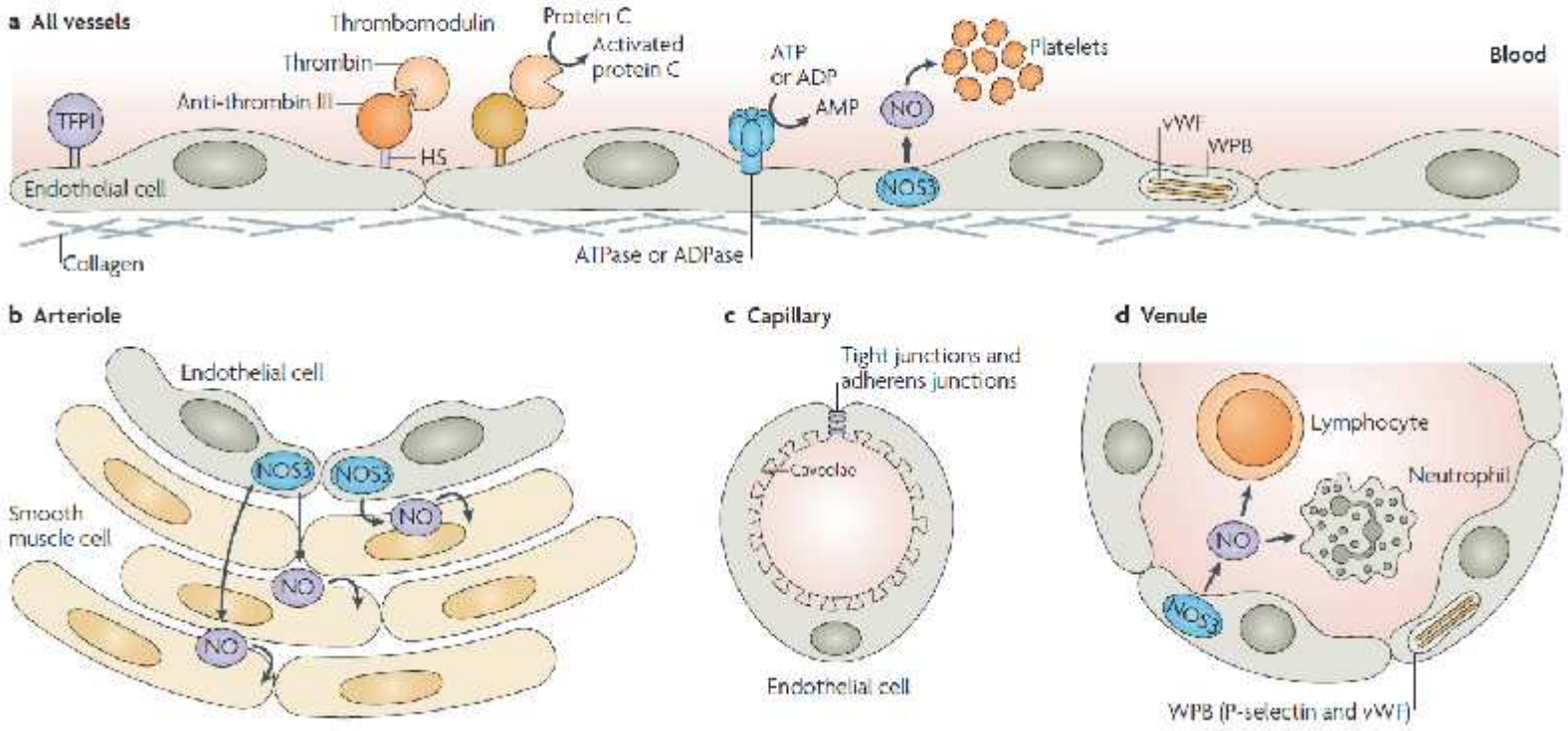
Endothelium





Scientific Reports 2018

Basic function of the Resting Endothelium



Inhibition of coagulation

Regulation of blood flow

extravasation of plasma proteins

leukocyte trafficking

Nature Medicine 2013

Activated endothelium

Type I activation

- G-protein coupled receptor (GPCR) signaling (eg through histamine) & activation of COX1 mediated signaling regulates vasodilation
- Immediate early response (lasts 10-20min). Desensitization of receptors to prevent restimulation.
- Transient response for limiting blood flow and the neutrophil extravasation (>>soft and transient oedema)

Activated endothelium

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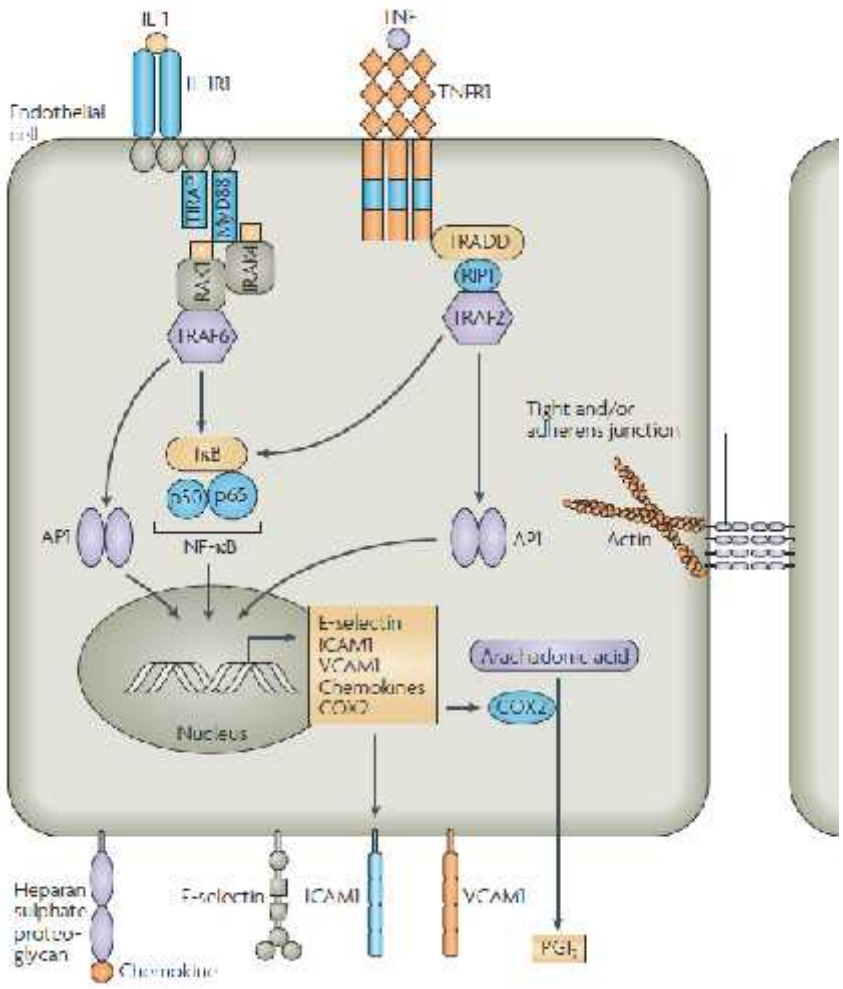
- Immediate early response (lasts 10-20min). Desensitization of receptors to prevent restimulation
- Transient response for limiting blood flow and the neutrophil extravasation (>>soft and transient oedema)

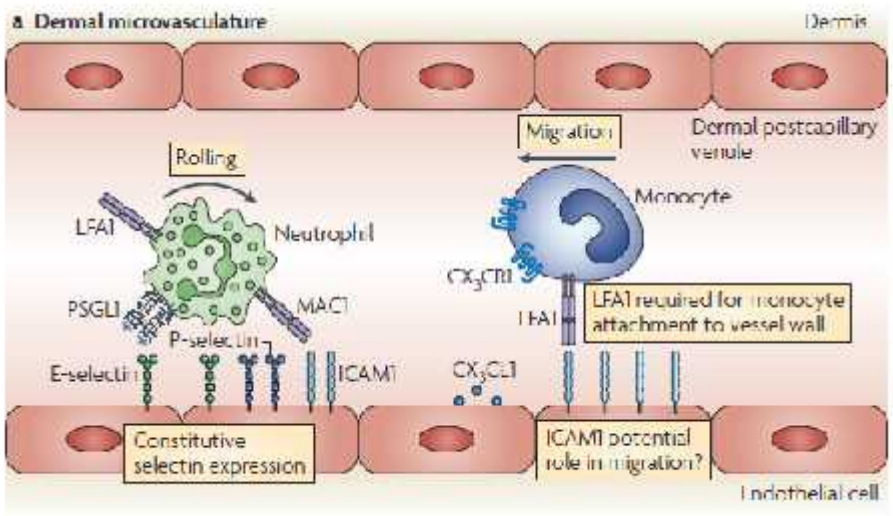
Type II activation

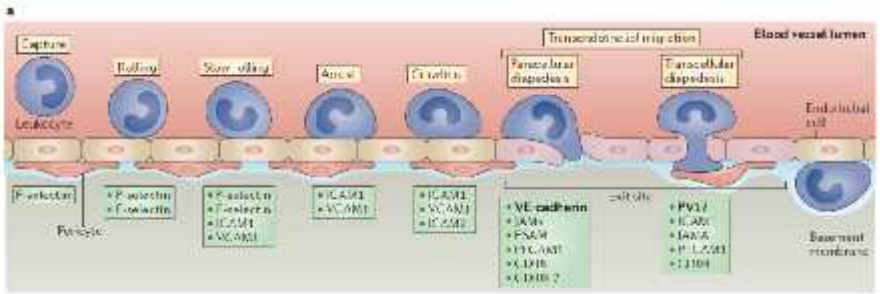
- more sustained inflammatory response
- requires transcription /translation of new proteins
- increased blood flow, increased vascular leakage of plasma proteins, and increased leukocyte recruitment at the site of inflammation
- COX2 mediated
- Hard swelling (leakage of very large plasma proteins such as fibrinogen, which is converted into a fibrin-rich clot



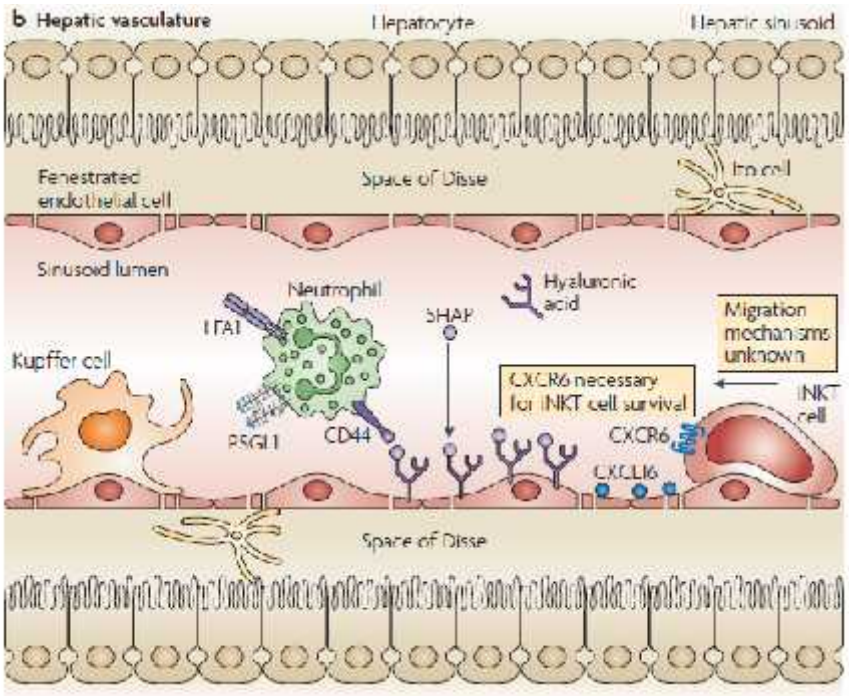
Nature Reviews Immunology 2007

Cellular and molecular interactions for immune surveillance and recruitment in the vasculature





Nature Reviews Immunology 2015



Nature Reviews Immunology 2009

Innate, sentinel-like characteristics of endothelial cells

Primary (not passaged) endothelial cells from various organs do express

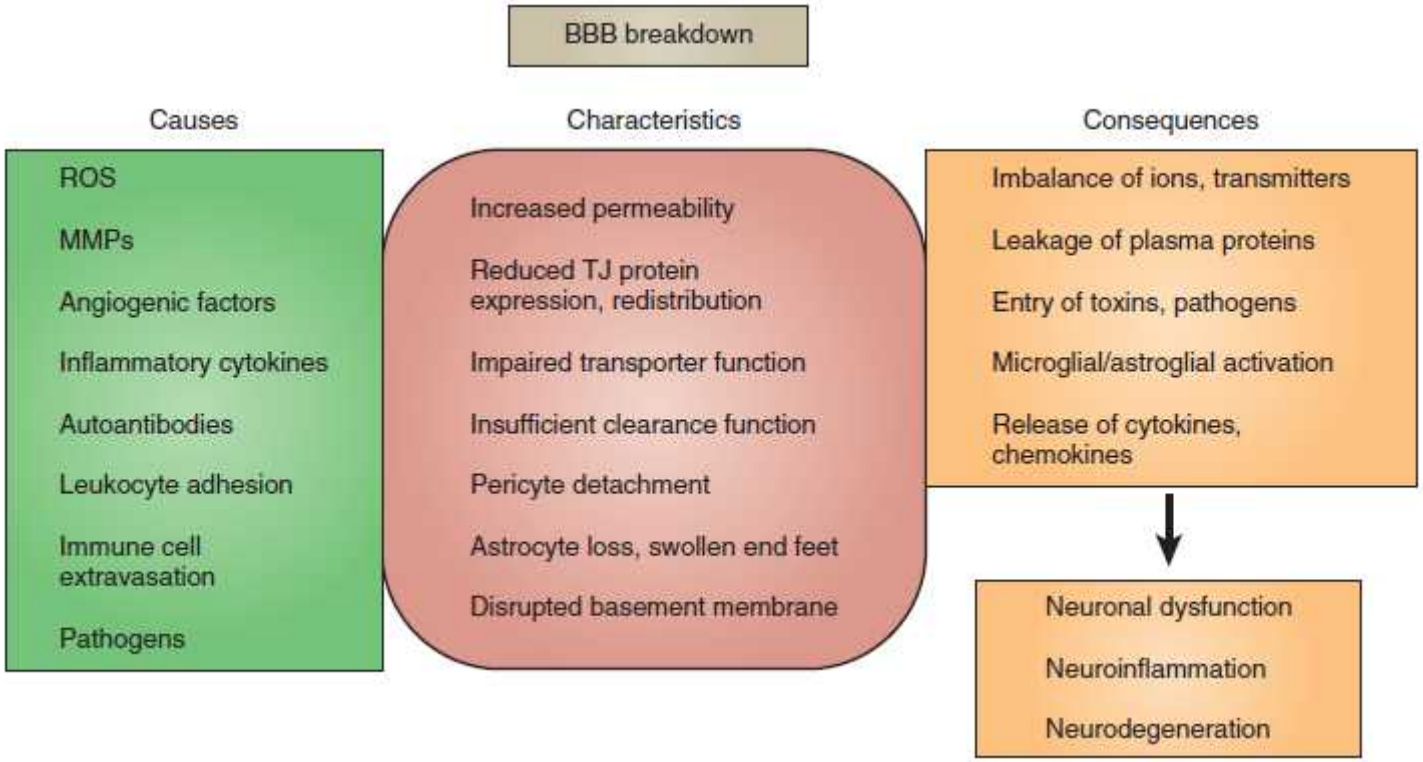
- TLR2/4/9
- CD14
- mD2 (also known as Ly96)
- MyD88 (TLR signalling adaptor myeloid differentiation primary-response protein 88)

Endothelial cells in adaptive immune responses

- Expression of MHCI and MHCII molecules and costimulatory ligands, endothelial cells could participate in adaptive immune responses (formation and activation of T cell memory)
- Polarization of adaptive immune responses:
  (a) in response to Th1 cell predominance, increased CXCR3 secretion sustains P selectin-mediated Th1 cell responses or
  (b) in response to Th2 cell predominance upregulated CCL26 expression sustains VCAM-mediated Th2 cell and eosinophil recruitment

Endothelial Tissue

Blood-Brain Barrier (BBB)



Nature Medicine 2013

Endothelial Tissue

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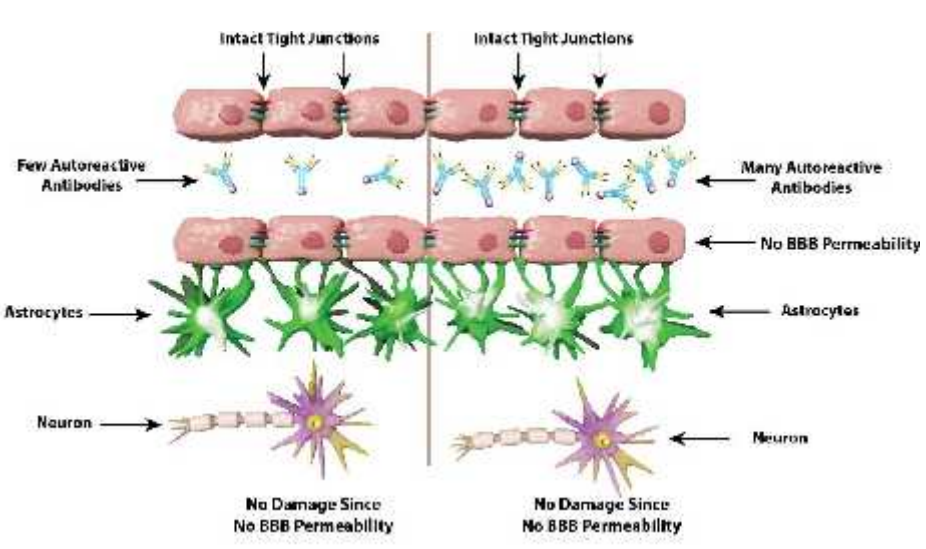
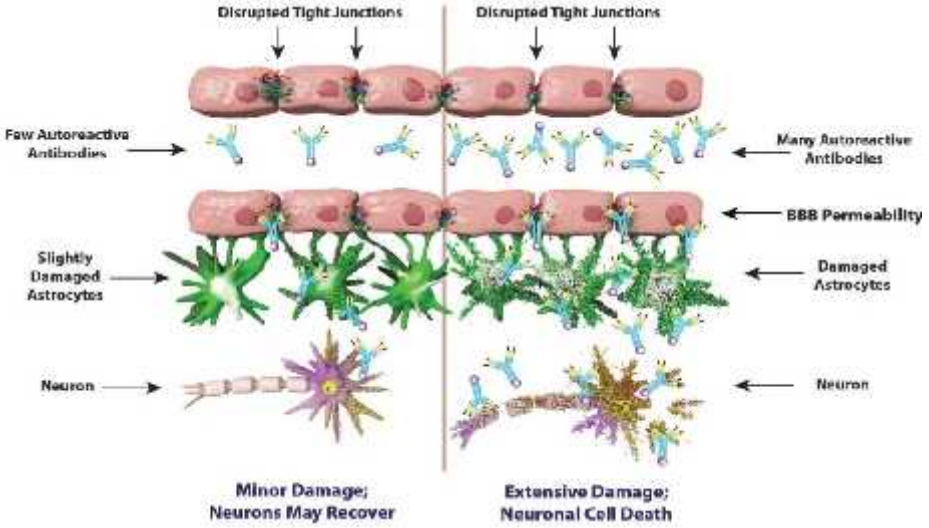


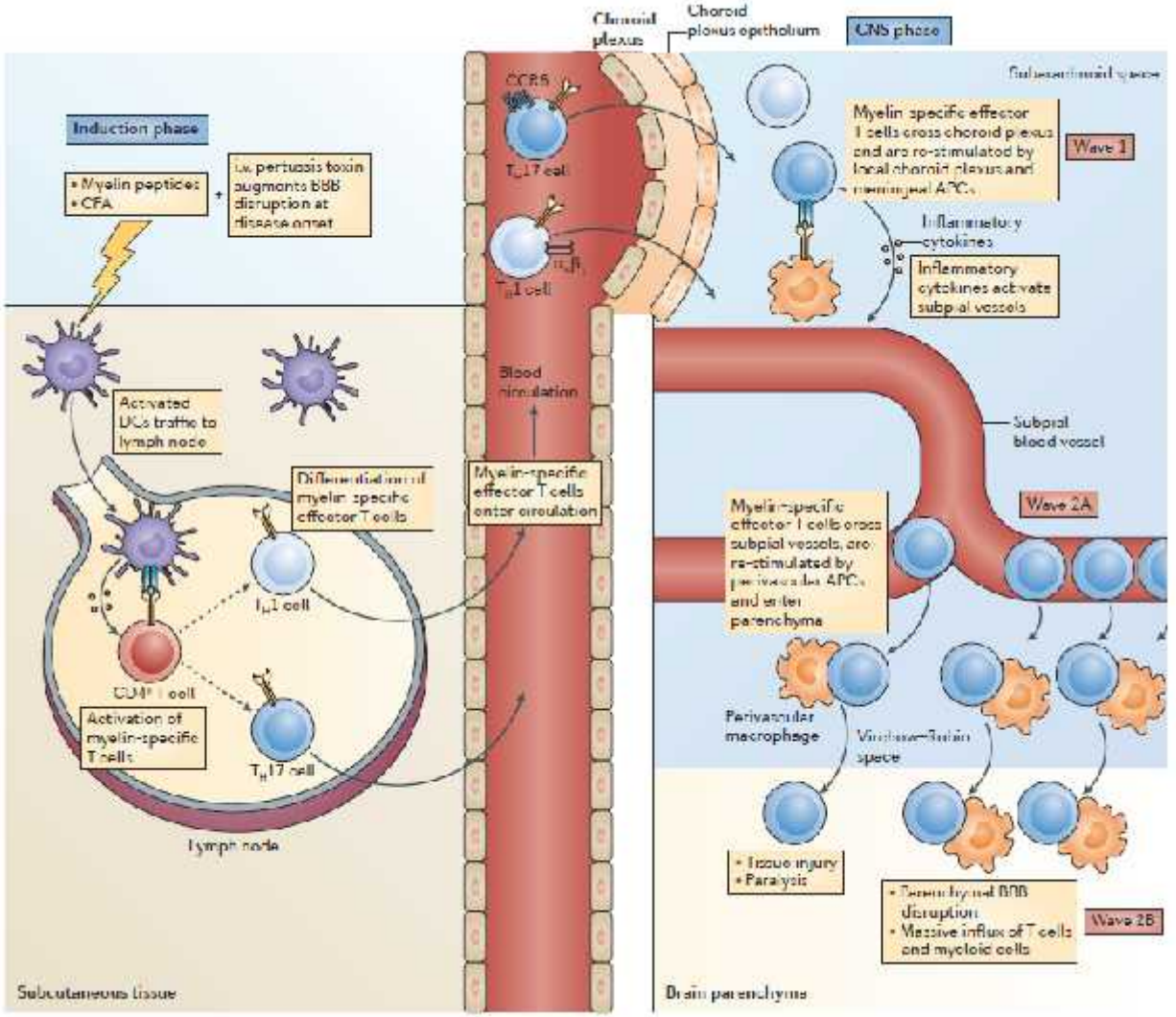


Endothelial Tissue

Blood-Brain Barrier (BBB)

The endothelium as a modulator of brain immune responses

Experimental Autoimmune Encephalomyelitis (EAE)



Nature Reviews Immunology 2012

Endothelium & Pericytes

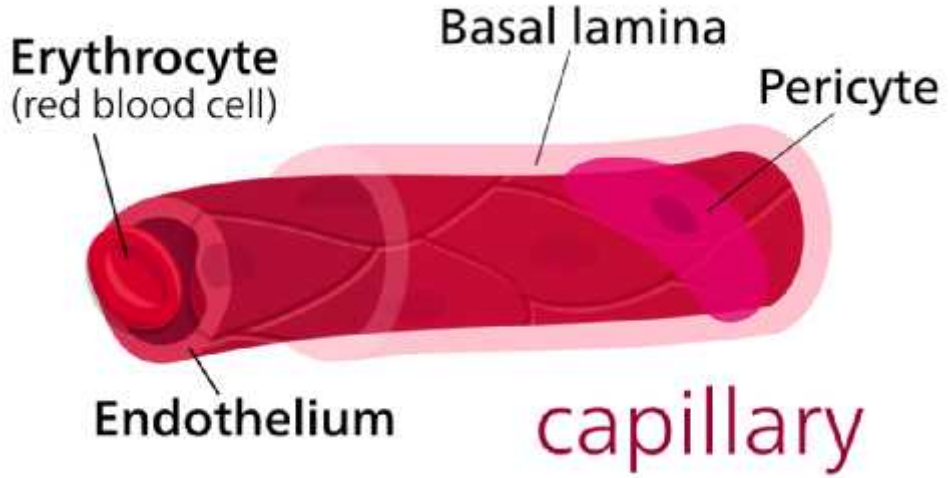
Endothelial cell–pericyte interactions lead to:

- Guidance of sprouts during angiogenesis
- Vessel stabilization by investment
- Induction of TJ formation
- Vessel maturation:

Negative regulation of endothelial cell proliferation

Tightening of interendothelial junctions

- Induction of basement membrane production by endothelial cells



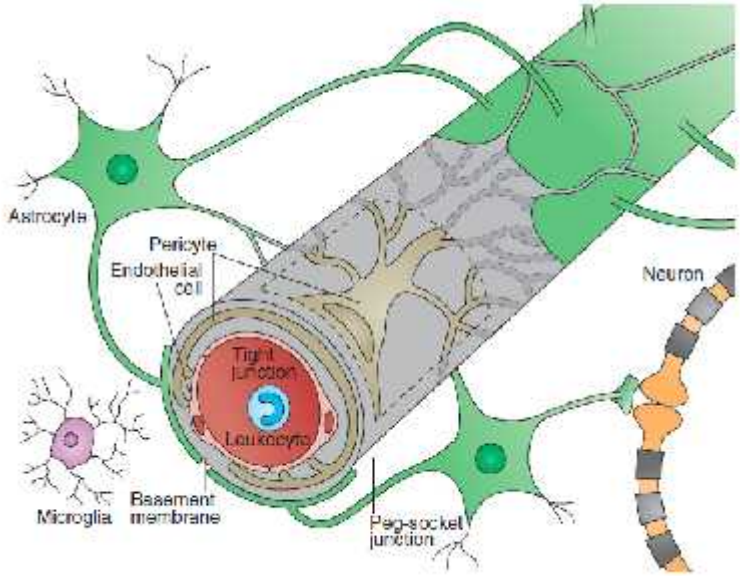
Endothelium & Pericytes

LETTER

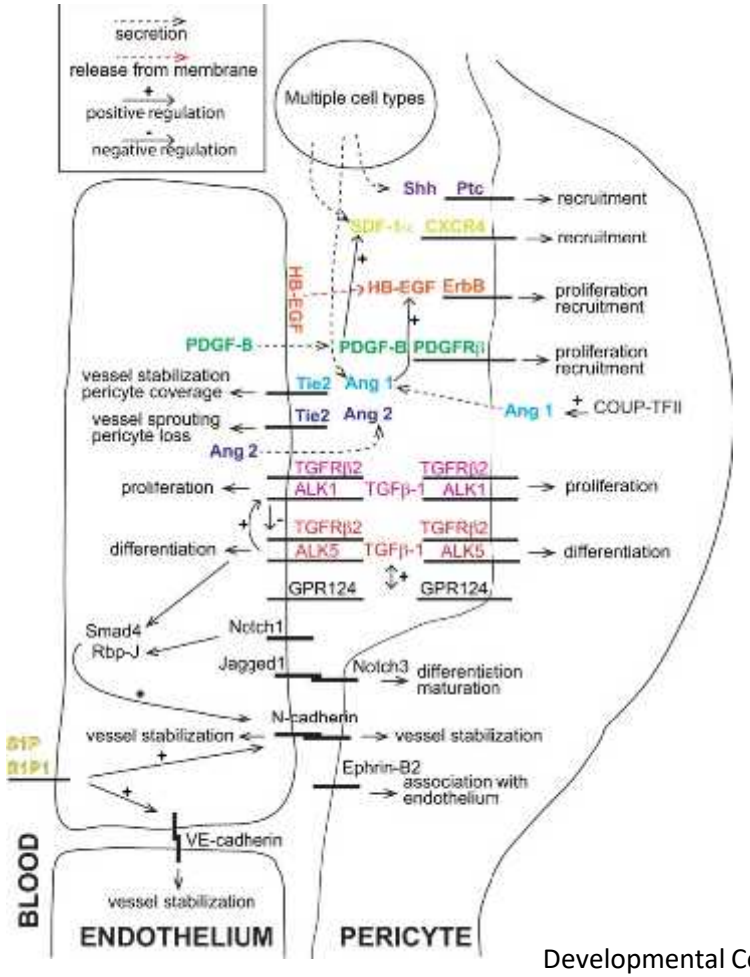
Nature 2010

Pericytes regulate the blood–brain barrier

Annika Armulik[1], Guillem Genové[1], Maarja Mäe[1], Maya H. Nisancioglu[1], Elisabet Wallgard[1], Colin Bianchi[1], Ingrid Hellström[1], Jonas Nordin[1], Per Lindblom[2], Karin Strittmatter[1,3], Bengt R. Johansson[2] & Christer Betsholtz[1]

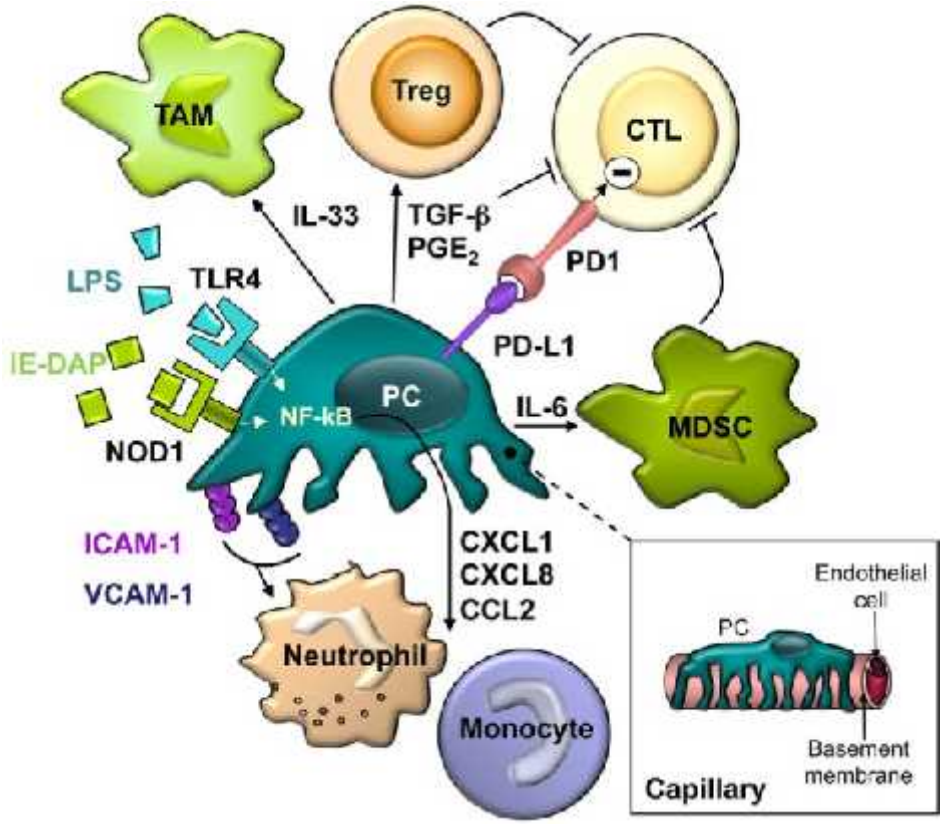


Nature Medicine 2013



Developmental Cell 2011

Immune cell interactions with Pericytes



Frontiers in Immunology 2016

Connective tissue

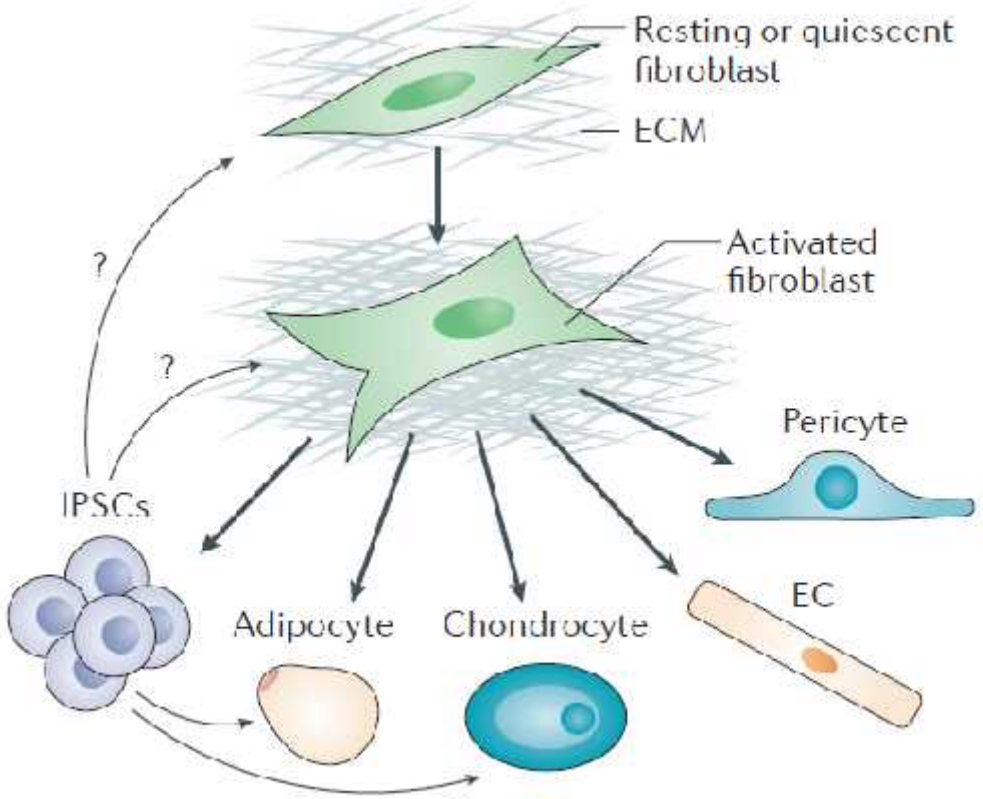
A type of tissue found in animals whose main function is to bind, support, and anchor the body.

Cells of mesenchymal origin: Fibroblasts, osteoblasts, chondrocytes, pericytes, etc

- **fibroblast**: A type of cell that synthesizes most of the extracellular matrix components.

extracellular matrix: Cells of the connective tissue are suspended in a non-cellular matrix that provides structural and biochemical support to the surrounding cells.

Plasticity of fibroblasts



The plastic nature of fibroblasts may also contribute to their functional heterogeneity

ECM

The ECM is well known for its ability to provide structural support for organs and tissues, for cell layers in the form of basement membranes, and for individual cells as substrate for cell motility.

“ECM-affiliated” proteins: mucins, secreted C-type lectins, galectins, semaphorins, and plexins and certain other groups of proteins that plausibly do associate with the ECM but are not commonly viewed as ECM proteins

ECM

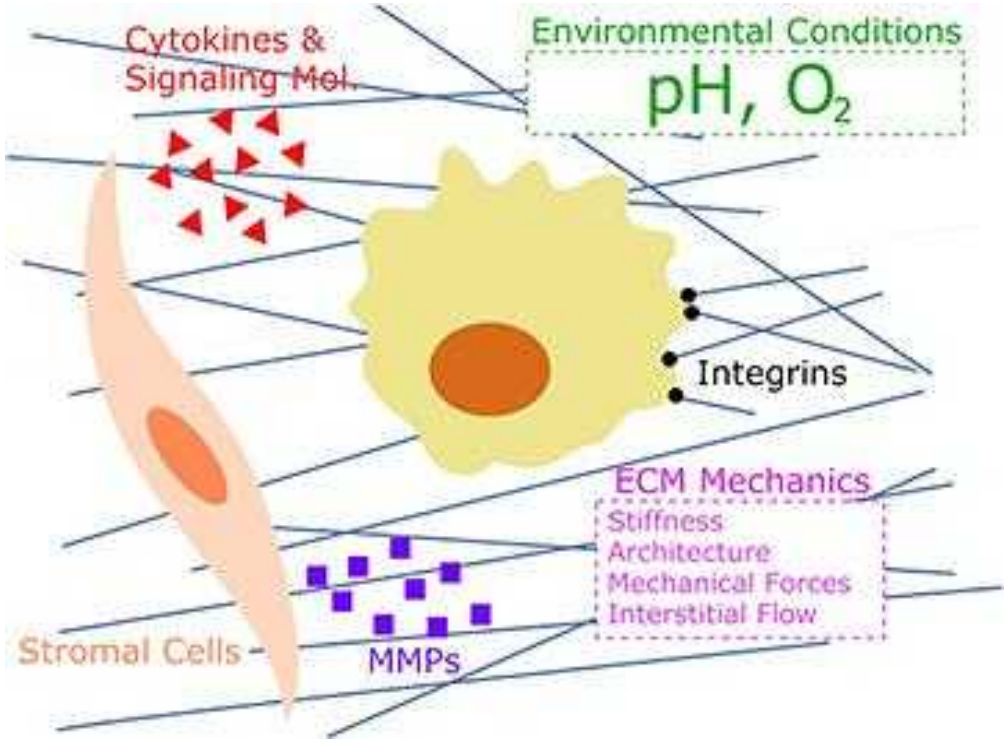
Matrisome

It comprises 1%–1.5% of the mammalian proteome (without considering the contribution of alternatively spliced isoforms (prevalent in transcripts of matrisome genes)

This list comprises almost 300 proteins, including 43 collagen subunits, 36+ proteoglycans, and around 200 glycoproteins.

ECM

mechanics



Convergent Science Physical Oncology 2017
Issue on the Mechanobiology and Biophysics of Cancer

Fibroblastic cells of immune system organs

Subsets of Fibroblastic Reticular Cells (FRCs) reported in lymphoid tissues

Name	Defining features	Defining functions
T cell zone reticular cells	• PDPN$^+$desmin$^+$ MADCAM1$^-$ • CCL19, CCL21 and IL-7 secretion	• Maintaining the T cell zone • Constructing the conduit network
Marginal reticular cells	• Subcapsular location • PDPN$^+$desmin$^+$MADCAM1$^+$IL-7^{hi} CXCL13$^+$RANKL^{hi} • Not found in tertiary lymphoid organs	• Rich source of IL-7 • Differentiation into FDCs
B cell zone reticular cells	• Resident cells: PDPN$^+$CCL19$^+$BAFF$^+$ and negative for FDC markers • Inducible cells: PDPN$^+$ subset of CD21$^-$ FRCs with a history of CD21 expression; convert into CXCL13$^+$ cells during the B cell response	• Maintaining B cell survival and follicle boundaries
FDCs	CD21$^+$CD35$^+$MFGE8$^+$CXCL13$^+$ICAM1$^+$ VCAM1$^+$BAFF$^+$	• Maintaining germinal centres • Facilitating the production of high-affinity antibodies
Pericytic FRCs	• PDPN$^+$ • Located around HEVs • PDPN signals to CLEC2 on platelets	Preventing bleeding from HEVs into lymph nodes

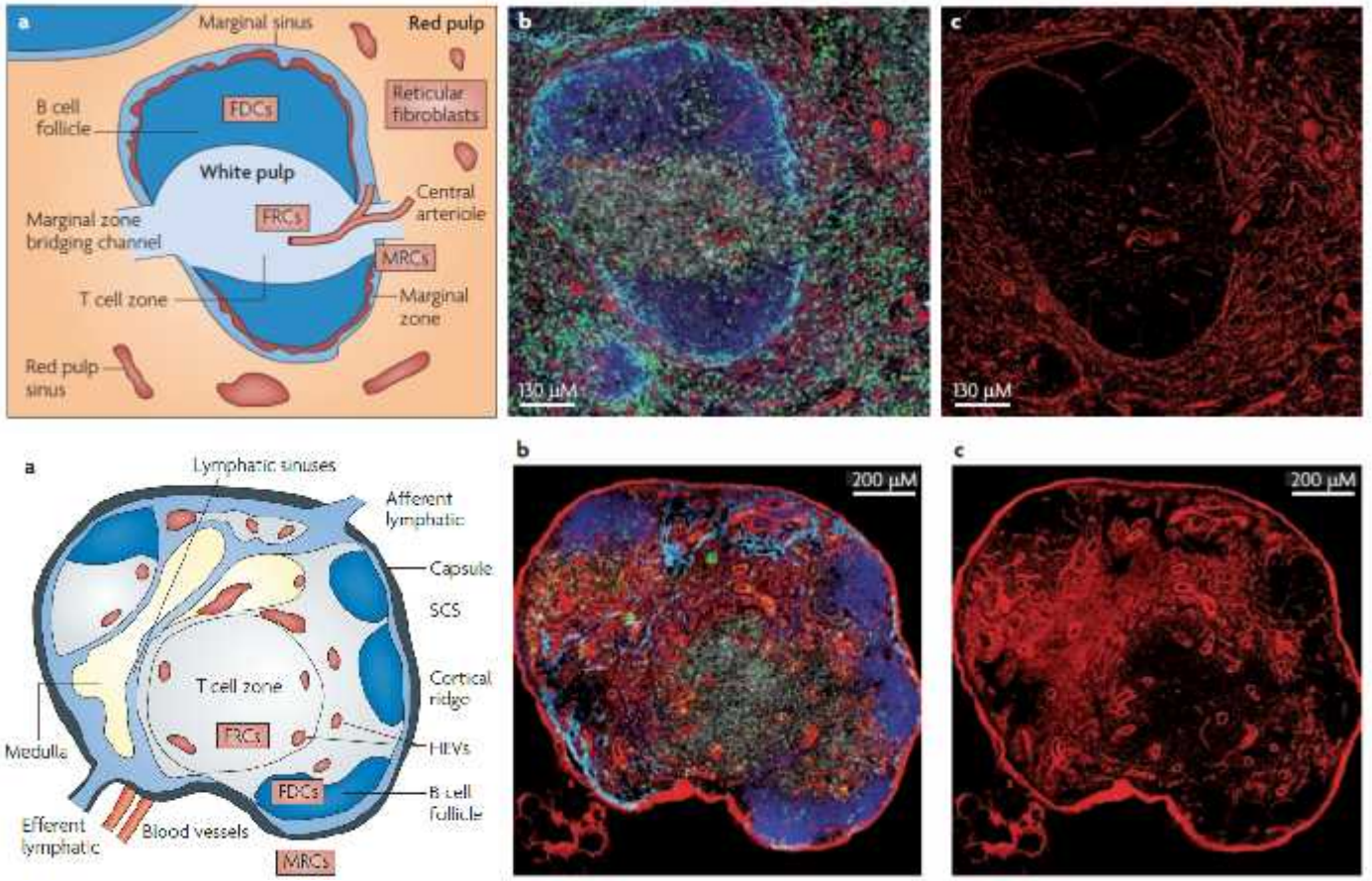
FRCs organize the lymph node microarchitecture

spleen

CD3+ T cells (white)
B220+ B cells (blue)
CD169+ marginal zone macrophages (cyan)
CD11c+ DCs (green)
ER-TR7+ stromal cells (red)

lymph nodes

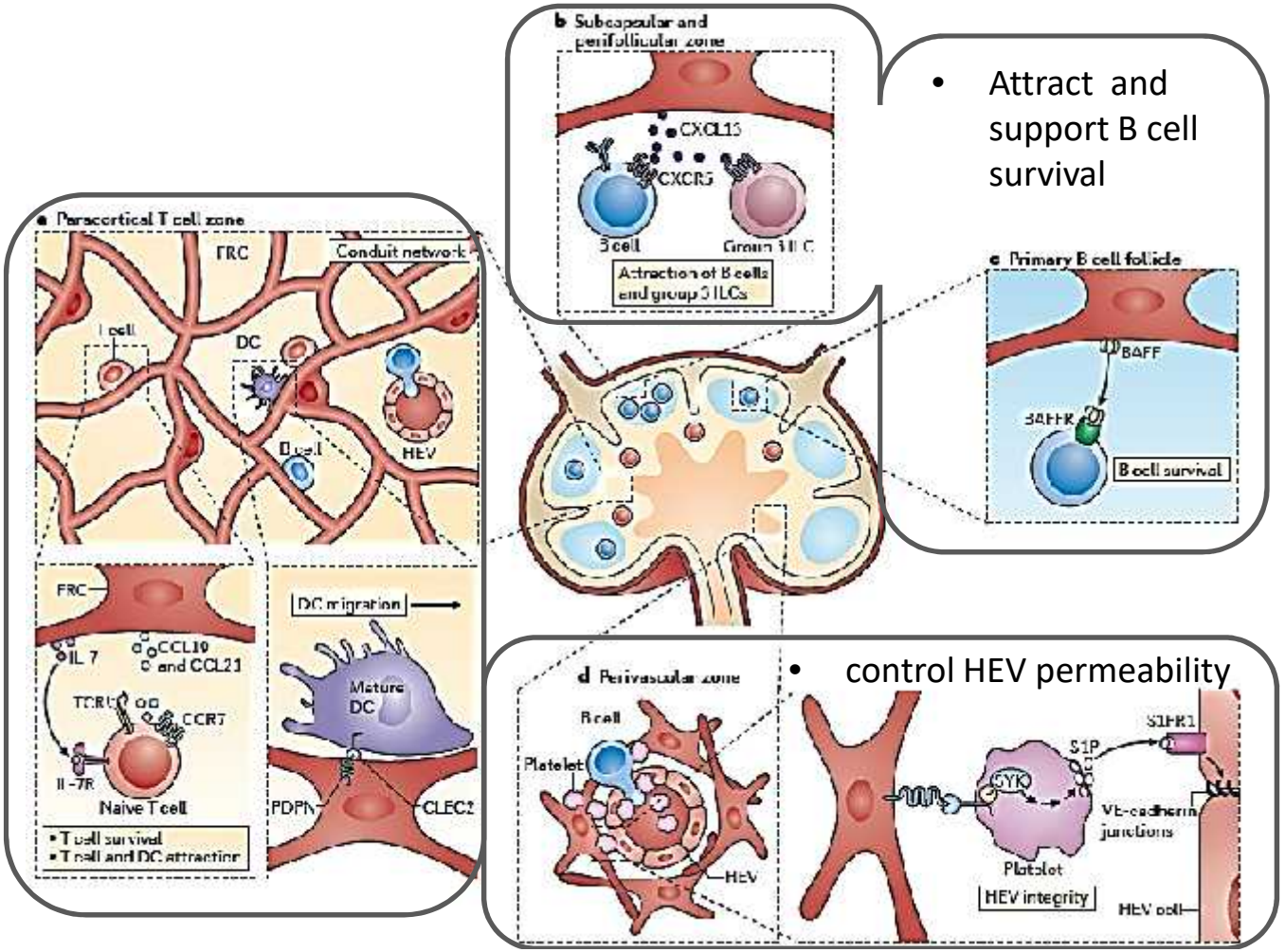
CD3+ T cells (white)
B220+ B cells (blue)
LYVE1+ lymphatics (cyan)
CD11c+ DCs (green)
PNAD+ HEVs (yellow)
ER-TR7+ stromal cells (red)



Nature Reviews Immunology 2009

FRCs organize the lymph node microarchitecture and function

- attract and maintain T cells
- mediate deletional tolerance
- suppress effector T cell proliferation
- maintain DCs and promote their migration



Nature Reviews Immunology 2015

Dynamic response of FRCs to infection

FRCs mediate lymph node flexibility

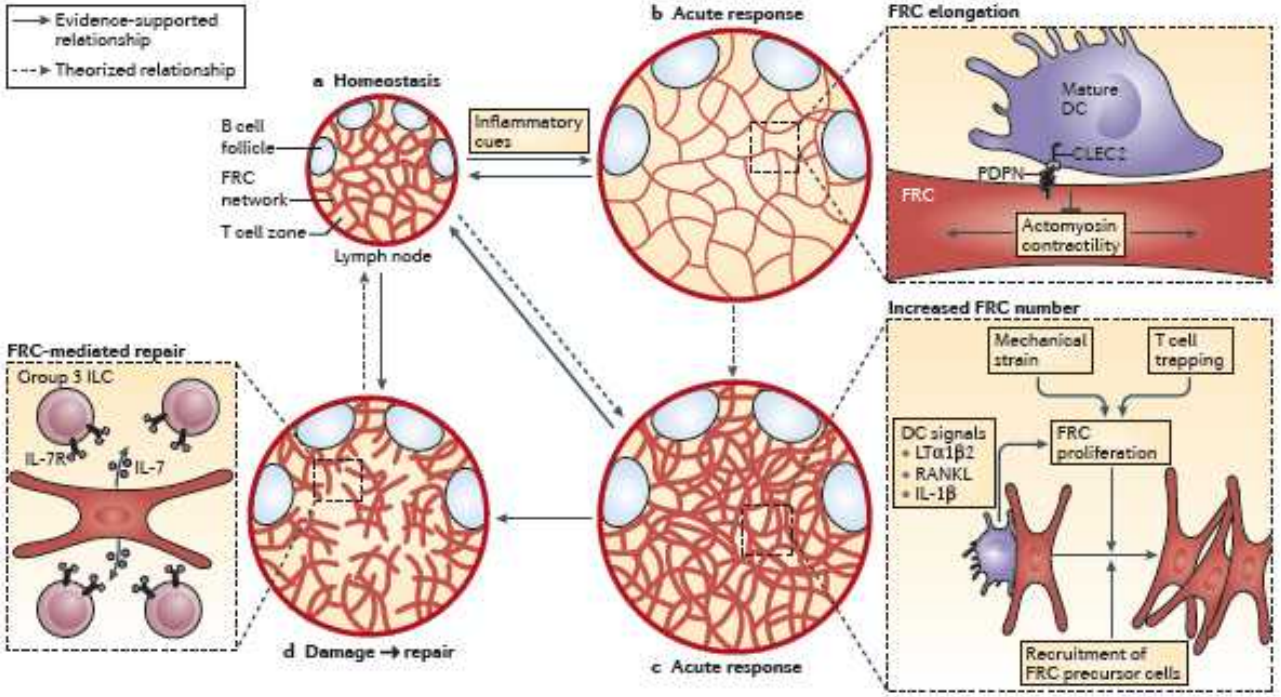
FRCs proliferate during infection

Loss of FRCs impairs systemic immune responses

FRCs are direct targets of virus infection (eg Ebola)

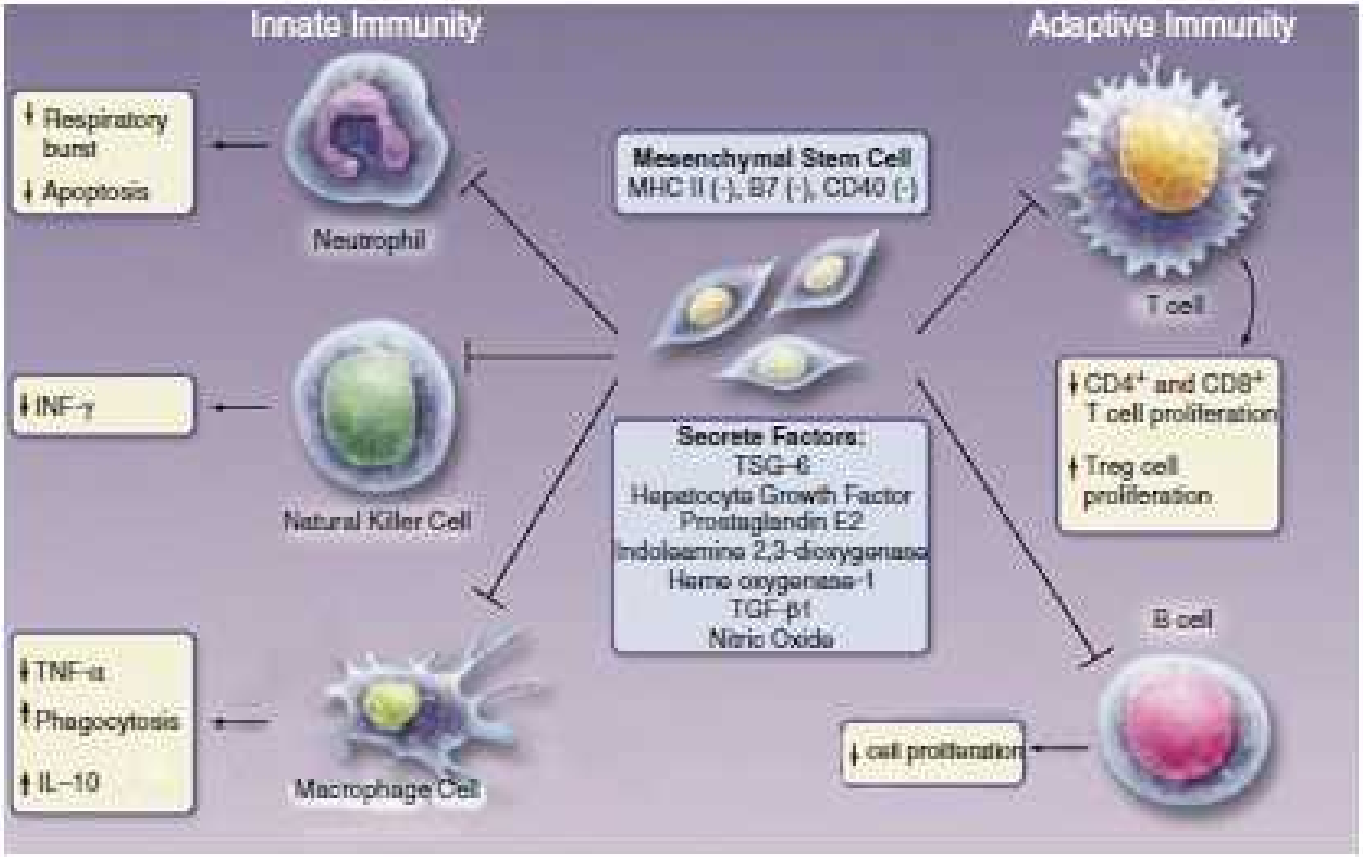
FRC-mediated lymph node fibrosis causes immunodeficiency.

FRCs are targets of allogeneic attack during GVHD



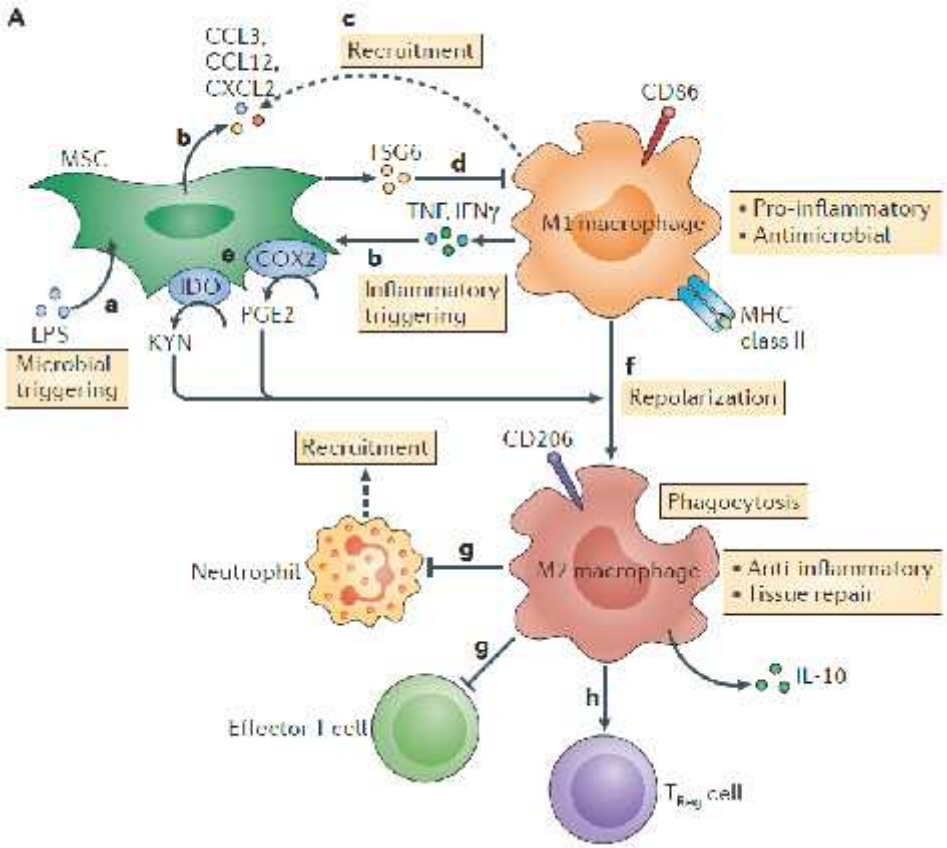
Nature Reviews Immunology 2015

Mesenchymal (stem) cells and immunomodulation



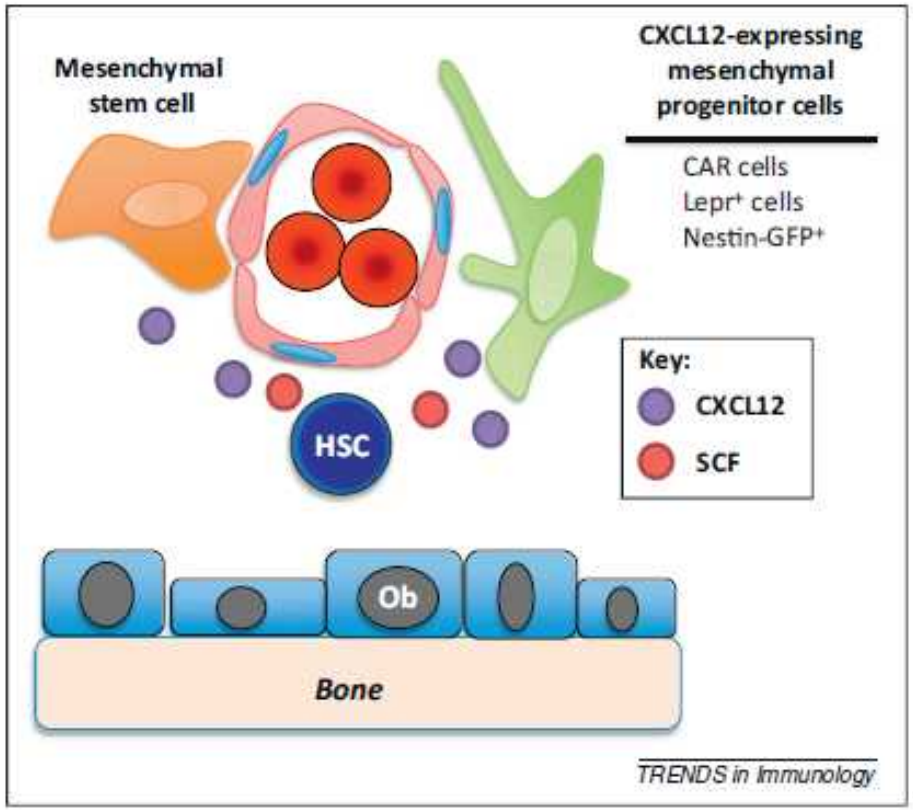
Advances in Stem Cell Research in Sepsis, 2019

Mesenchymal (stem) cells and immunomodulation

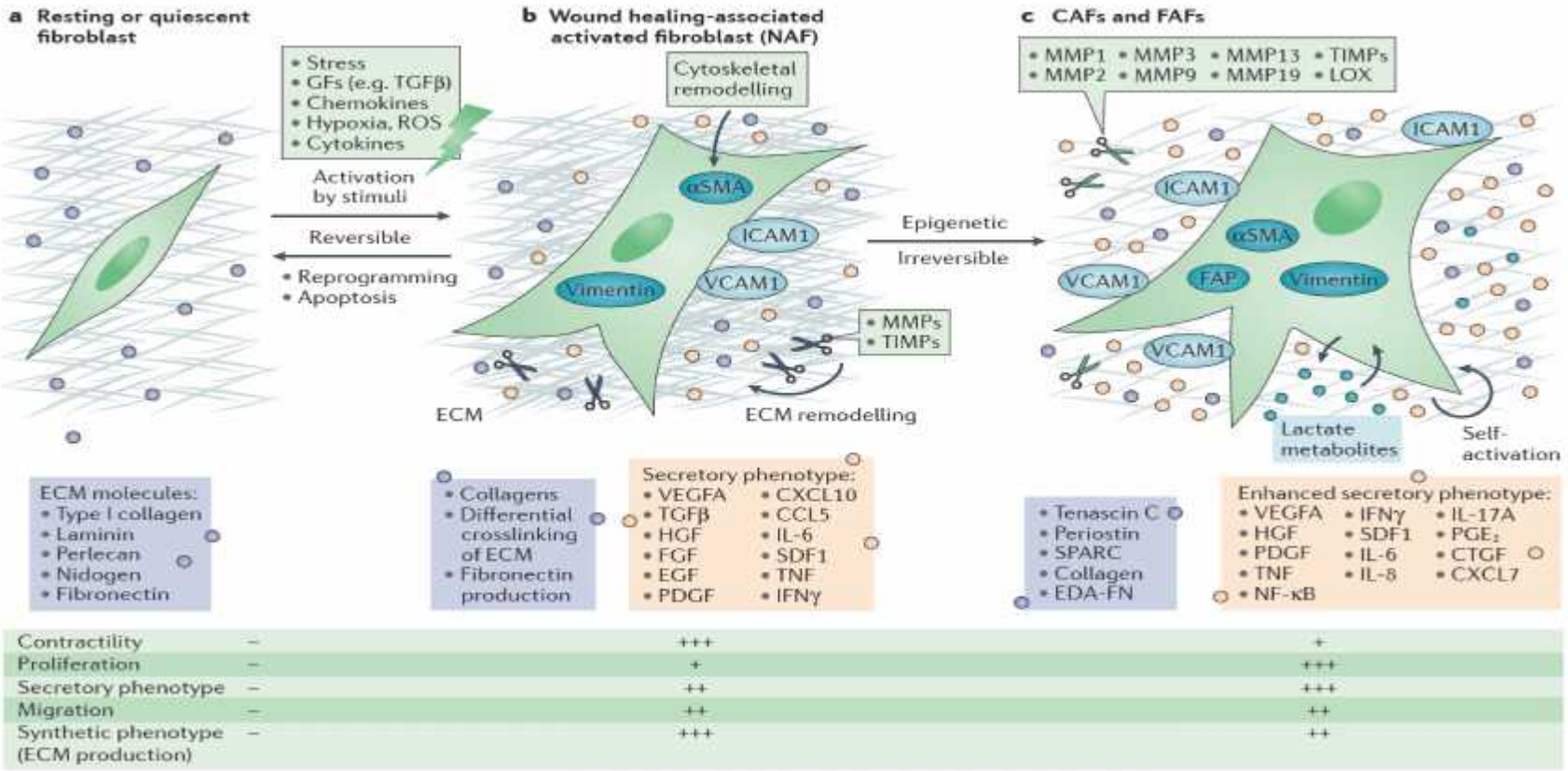


Nature Reviews Immunology 2013

BM stromal cells modulate hematopoiesis

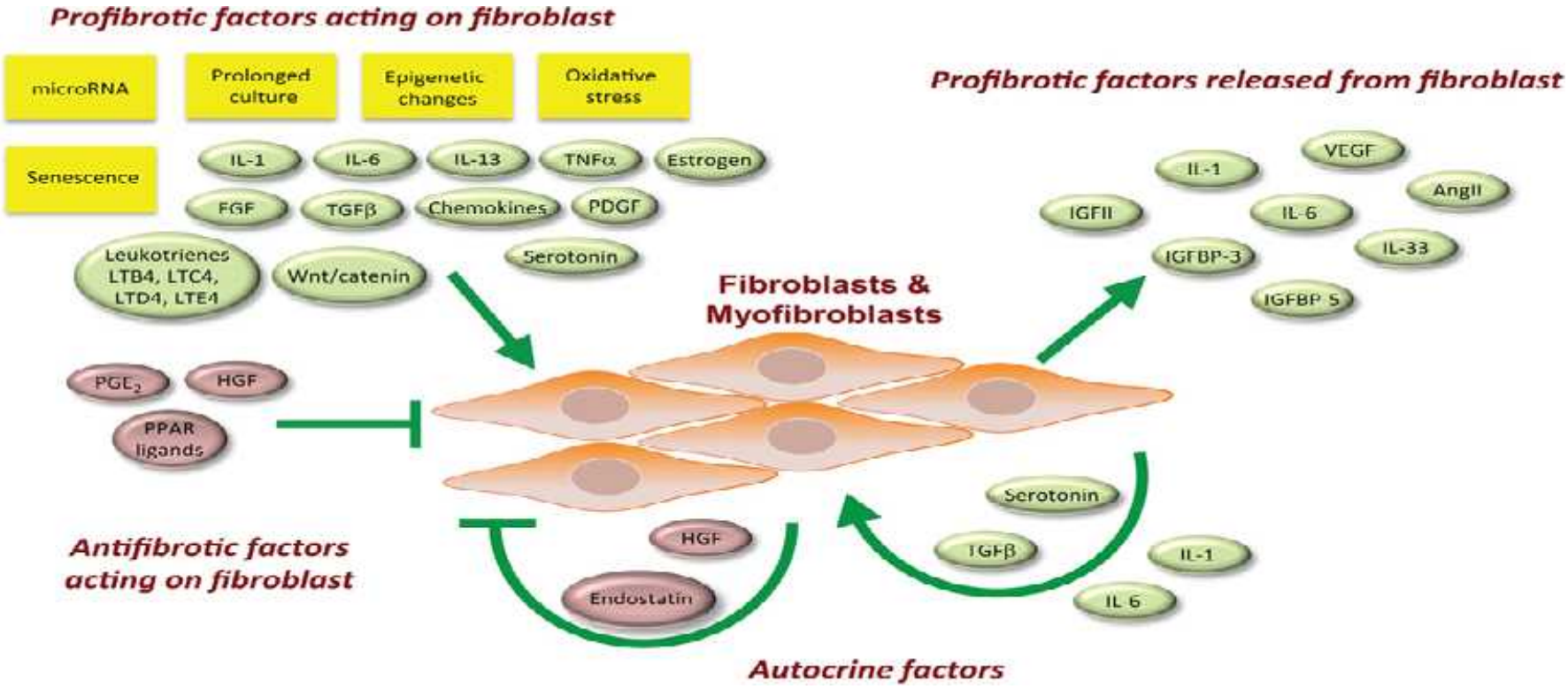


Fibroblast act directly and/or indirectly to modulate immune responses

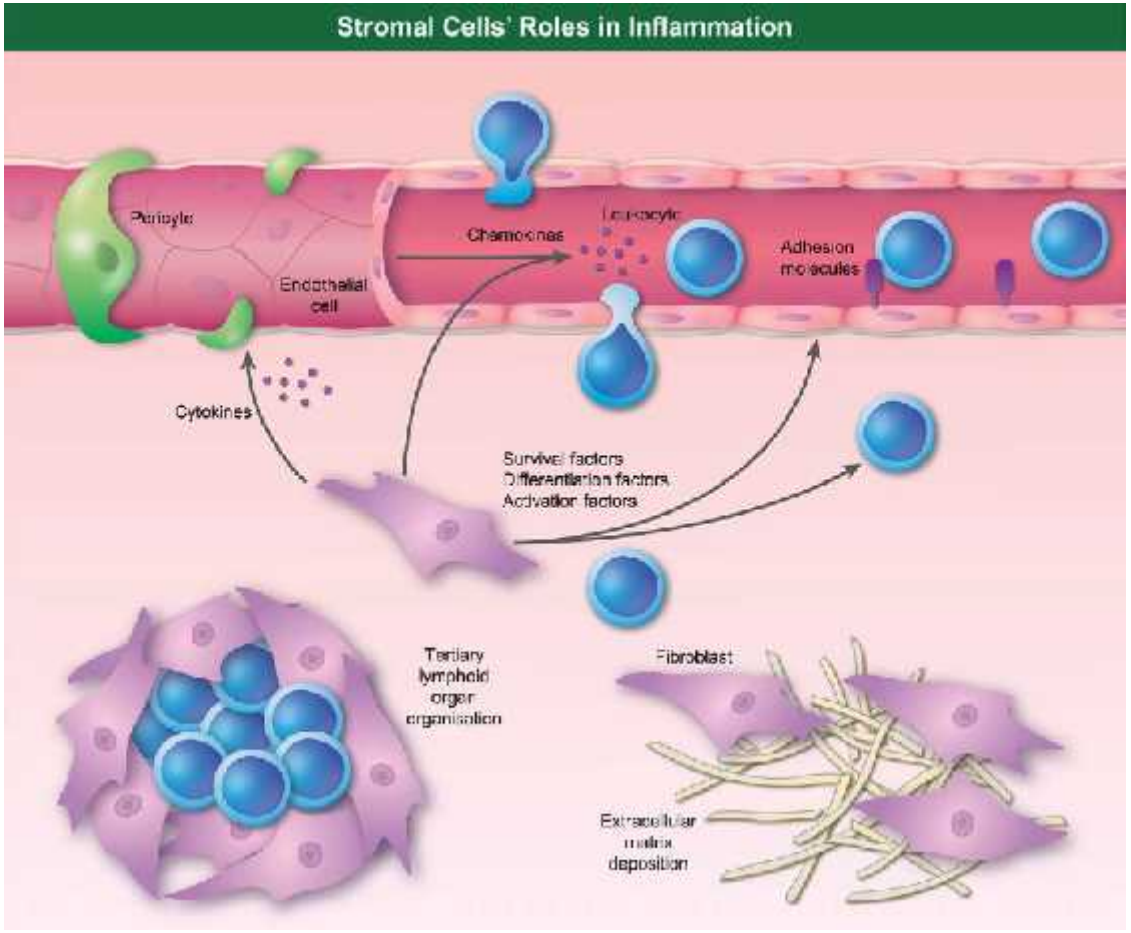


Nature Reviews Cancer 2016

Fibroblast act directly and/or indirectly to modulate immune responses



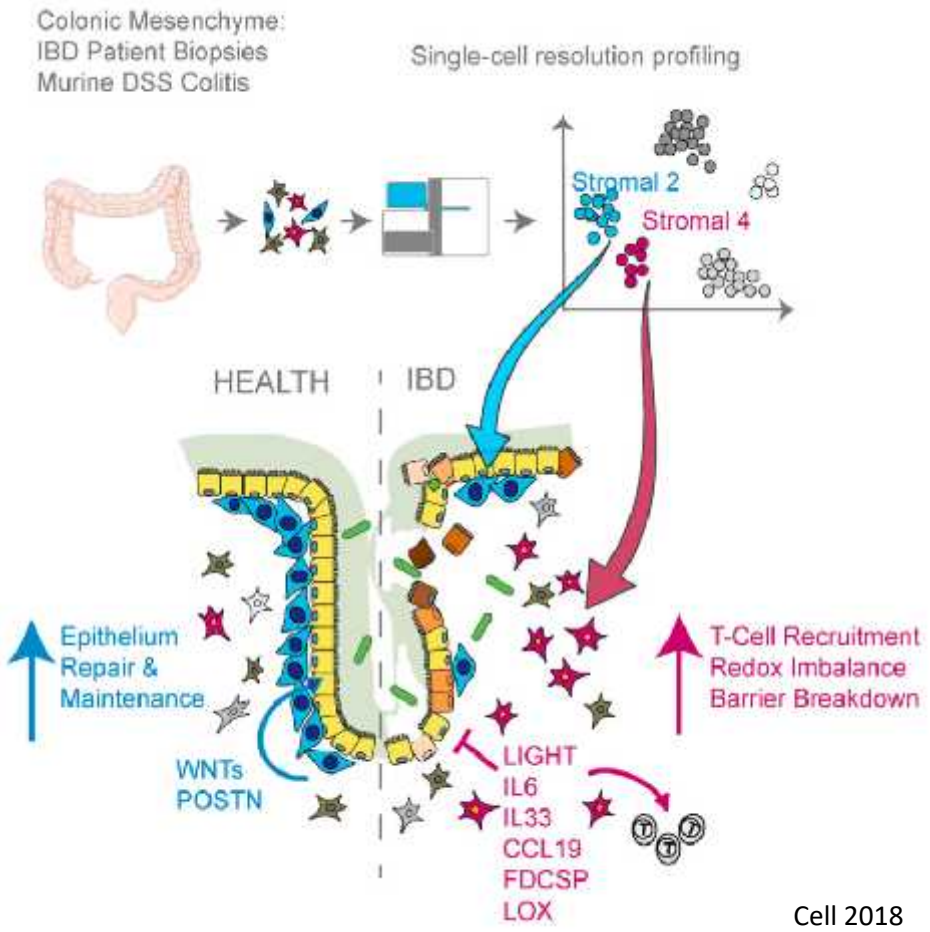
Frontiers in Pharmacology 2014



Clinical and Experimental Immunology 2018

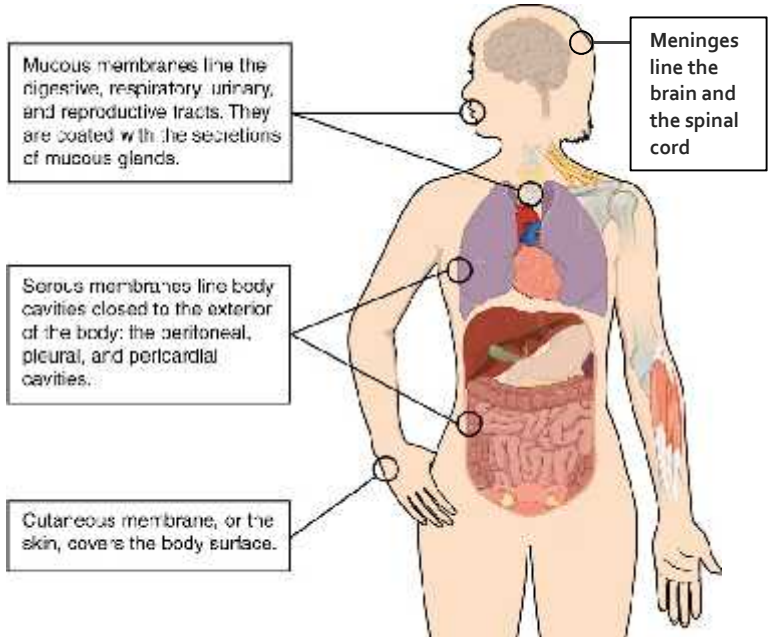
SC transcriptomics: mesenchymal subpopulations and their distribution in healthy and diseased intestine

IBD

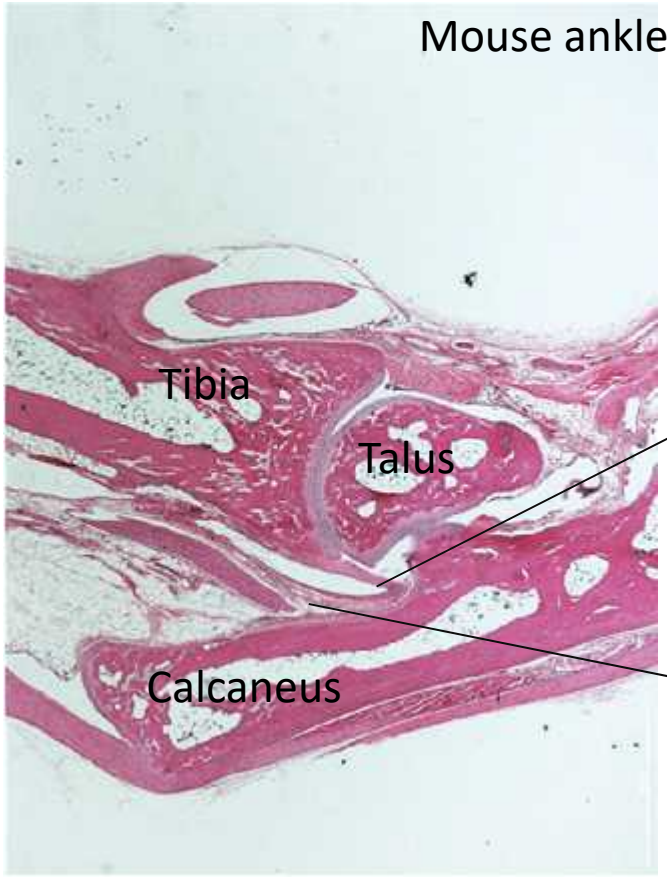


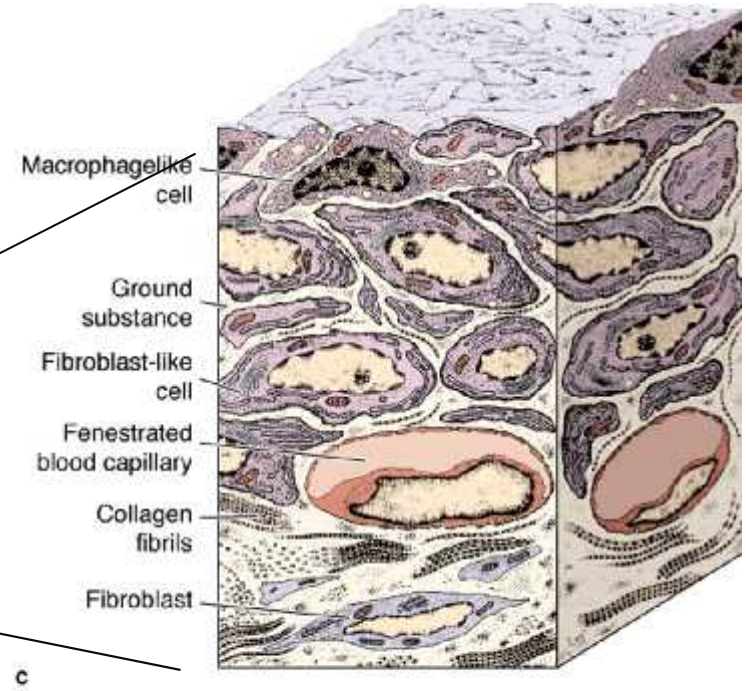
Cell 2018

Connective Tissue Membranes



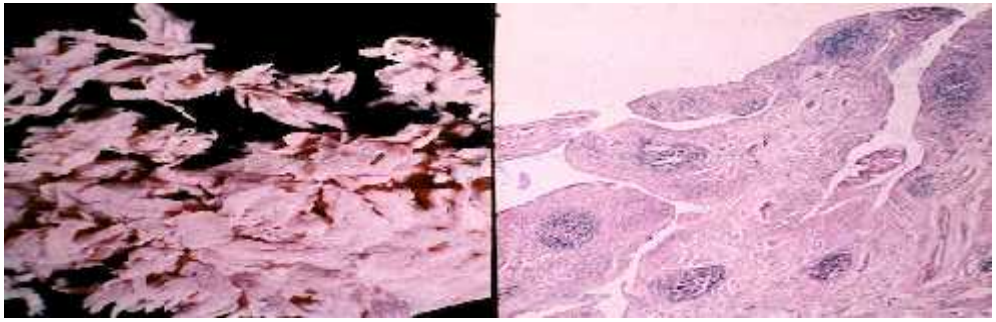
Normal Synovium



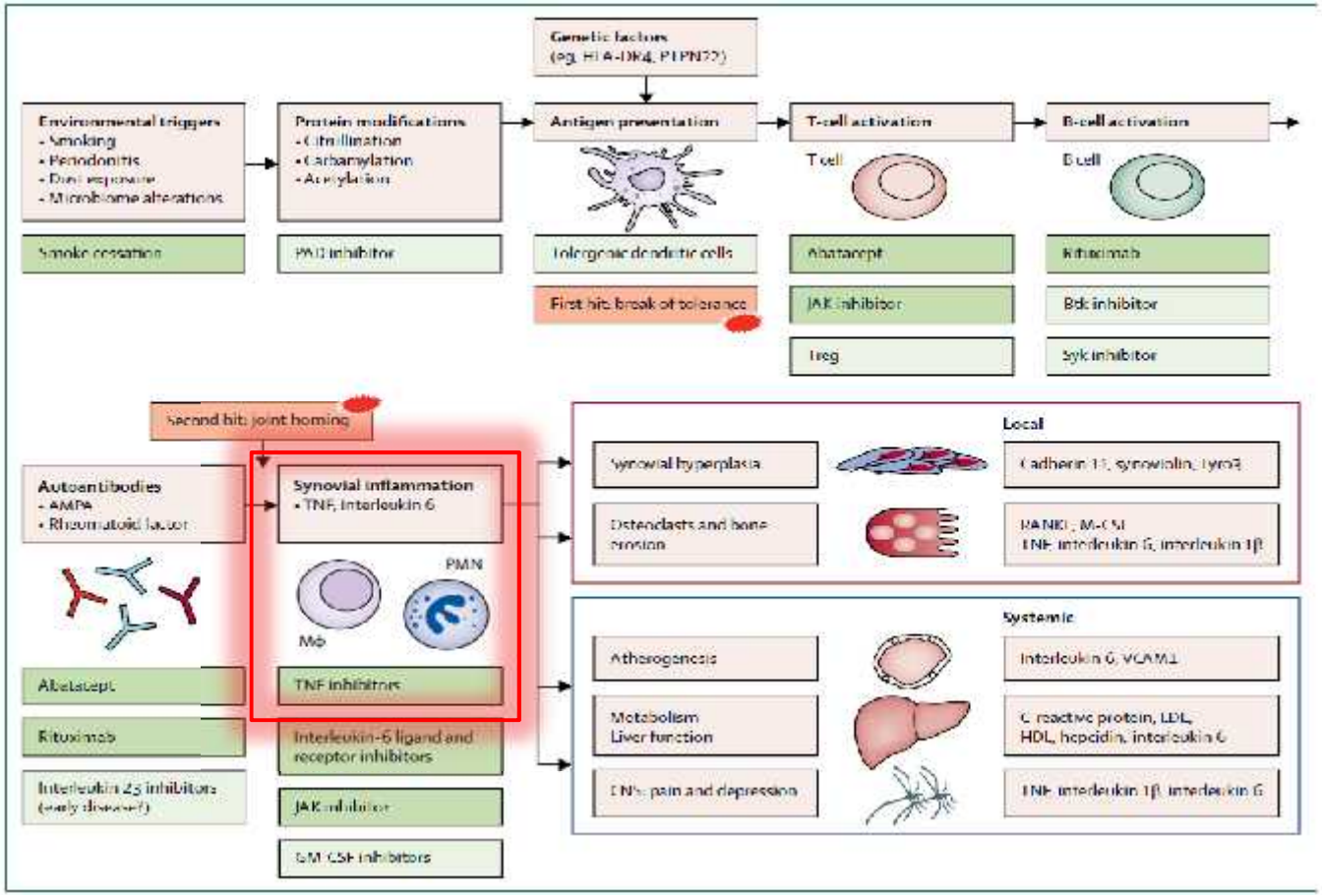


Source: Mescher AL: *Junqueira's Basic Histology: Text and Atlas, 12th Edition*: http://www.accessmedicine.com

Progressing Synovitis: a hallmark of RA



Rheumatoid Arthritis:
Pathophysiological pathways & therapeutic intervention strategies



pathophysiological steps in the development of RA

existing therapeutic intervention strategies

therapeutic interventions in development

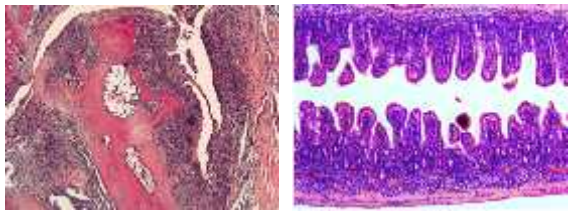
Lancet 2017

RA modeling in mouse and TNF

hTNFTg (Tg197)
(Keffer et al, EMBO J 1991)

Human TNF expression:
Thymus, Spleen, Kidney, Lung, Brain, Joints

- 100% phenotypic penetrance of Inflammatory polyarthritis
- Non-haemopoietic tissues express spontaneously high levels of huTNF



TNF$^{\Delta ARE}$
(Kontoyiannis et al, Immunity 1999)

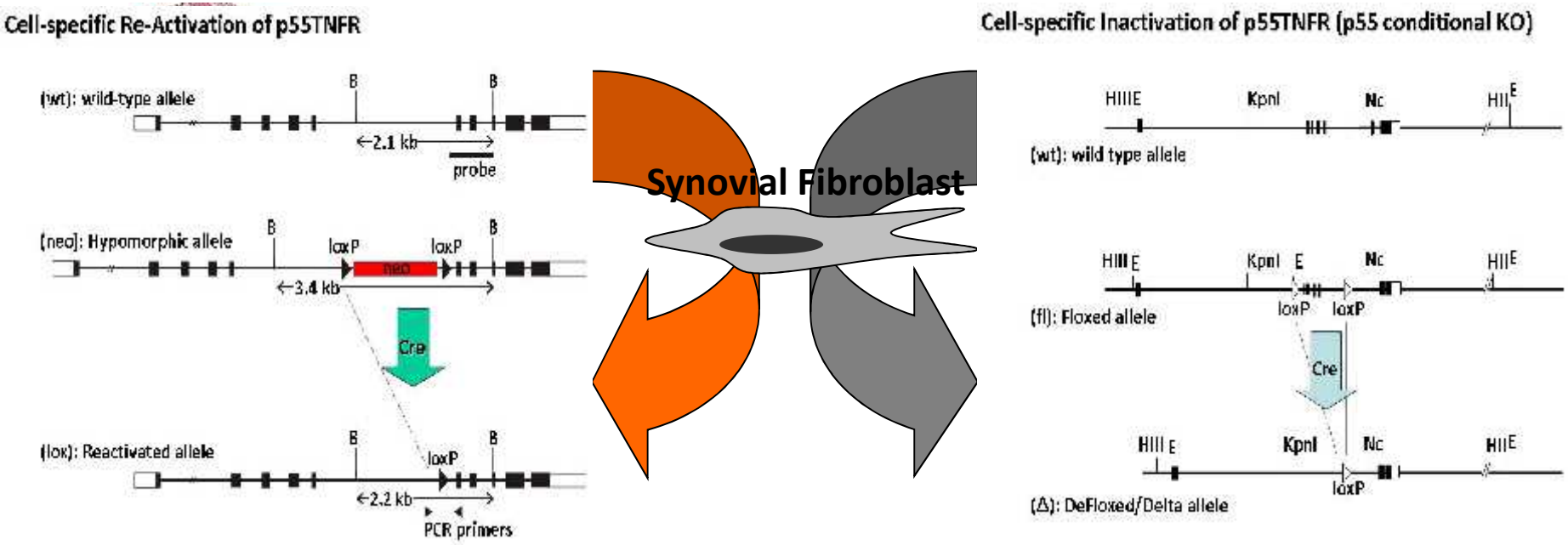
Loss of ARE elements leads to:

- Increased stability and translation of TNF mRNA
- 100% phenotypic penetrance of Inflammatory arthritic and intestinal diseases
- Chronic expression of TNF from haematopoietic cells and spontaneous ectopic expression of TNF from non-haemopoietic cells
- Loss of anti-inflammatory translational mechanisms

TNFRI(p55)-dependent phenotypes

T- and B-cell independent development of arthritic disease

Mesenchymal-specific p55TNFR signaling in TNF-mediated arthritis (mesenchymal or innate TNF stimuli):



Sufficient

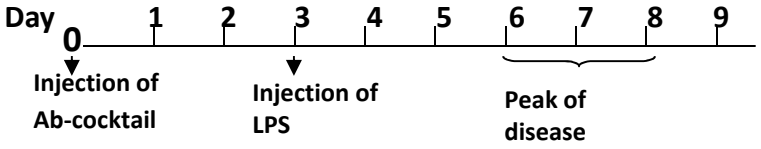
(JEM 2008)

…and Necessary

(Nat Commun 2018)

For disease development

Mesenchymal p55TNFR signals are necessary for the development of TNF-mediated arthritis (adaptive stimuli-CAIA model)



TNFRI (p55) dependent

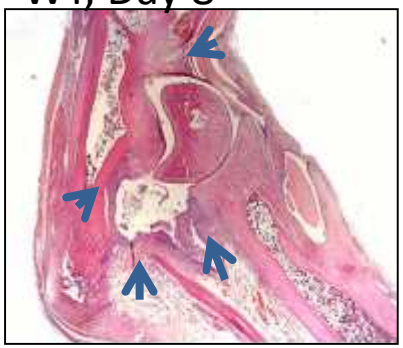
IL1 dependent

Complement dependent

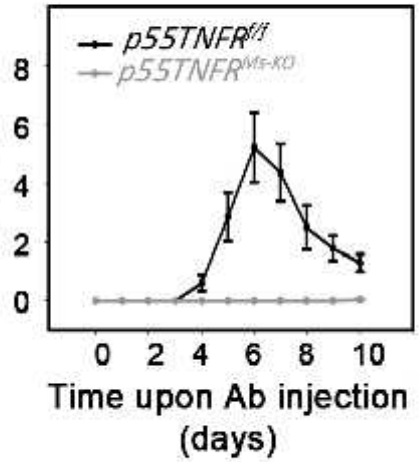
IL6 independent

T- & B-cell independent

WT, Day 8

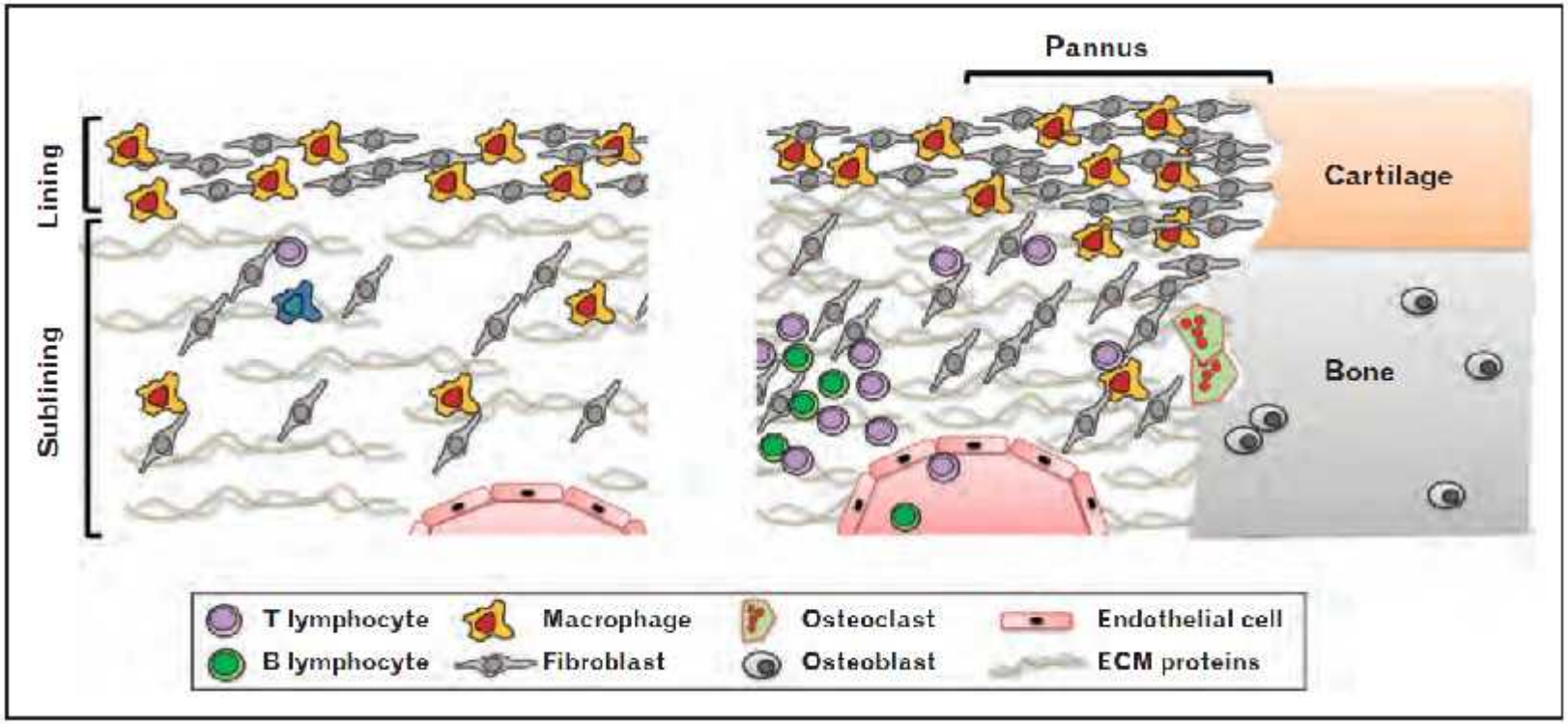


p55TNFR$^{SF\text{-}KO}$, Day 8



Nat Commun 2018

Rheumatoid arthritis: From synovitis to pannus formation

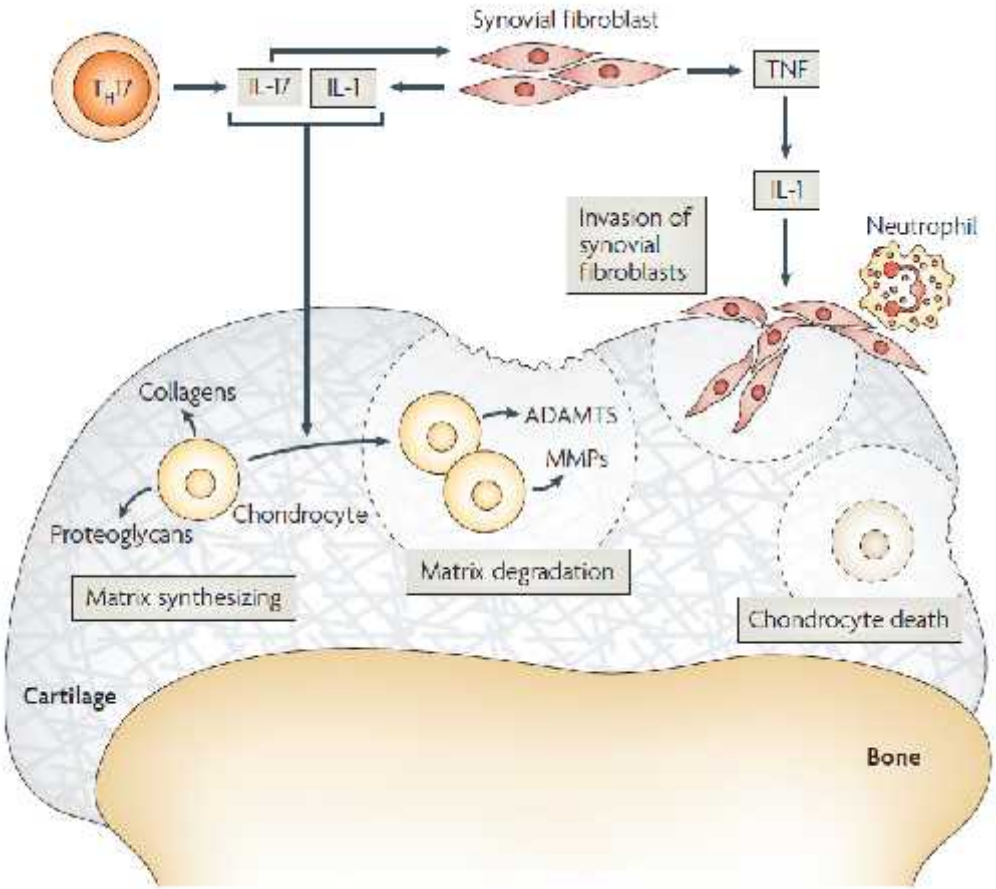


Curr Opin Rheumatol 2015

Pathogenic mechanisms leading to permanent destruction of joint architecture in RA

Regardless of the initial trigger

- leading role of SFs in joint remodeling



Nature Reviews Immunology 2007

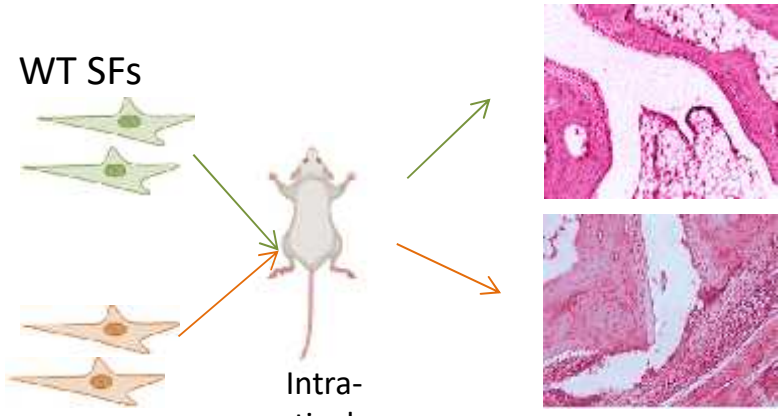
Why chronic inflammation persist?

Autonomous invasive properties of RASFs

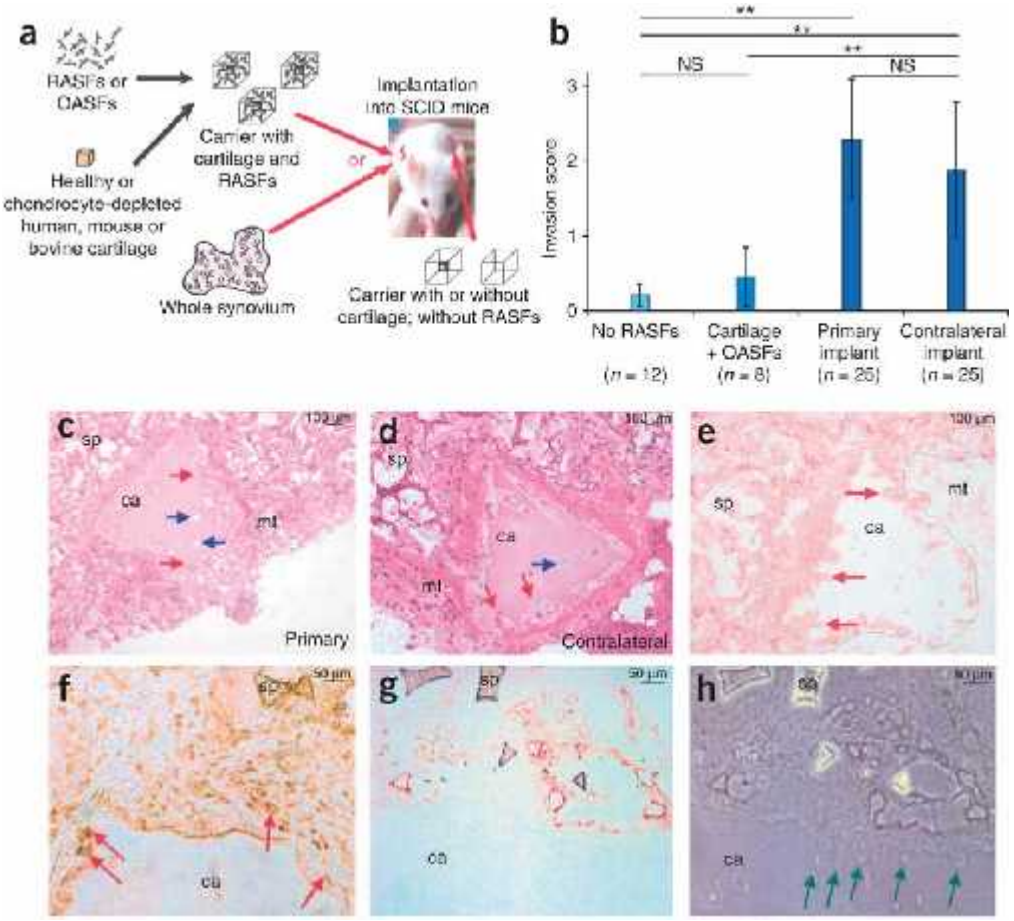
Research article
Open Access

Functional analysis of an arthritogenic synovial fibroblast

Vassilis Aidinis[1], David Plows[9], Sylva Haralambous[2], Maria Armaka[1], Petros Papadopoulos[1], Maria Zambia Kanaki[1], Dirk Koczan[3], Hans Juergen Thiesen[3] and George Kollias[1]



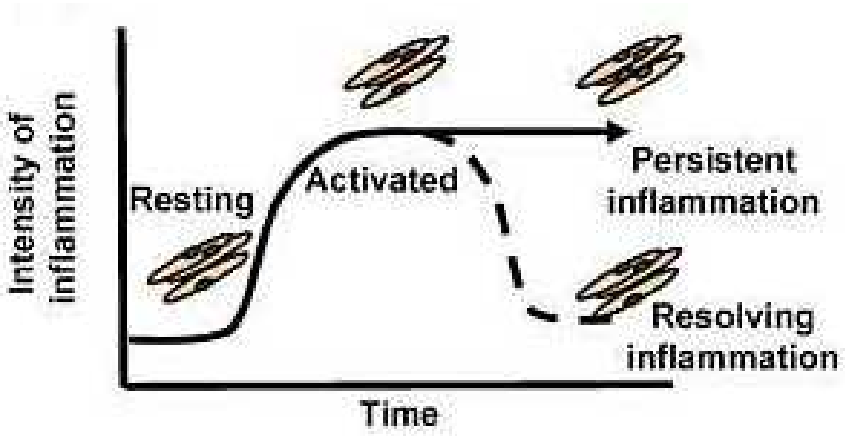
Arthritis Research and Therapy 2003

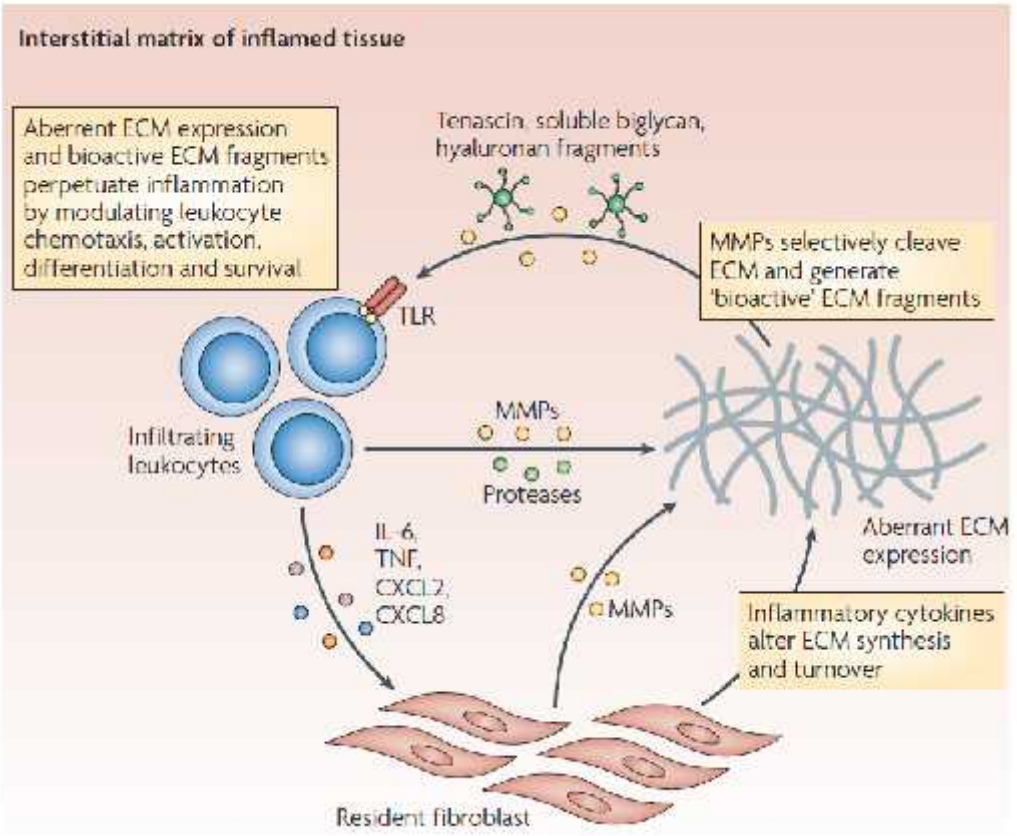


Nature Medicine 2009

Why chronic inflammation persist?

RA pathogenetic paradigm suggests
a mesenchymal-based hypothesis





Nature Reviews Immunology 2010

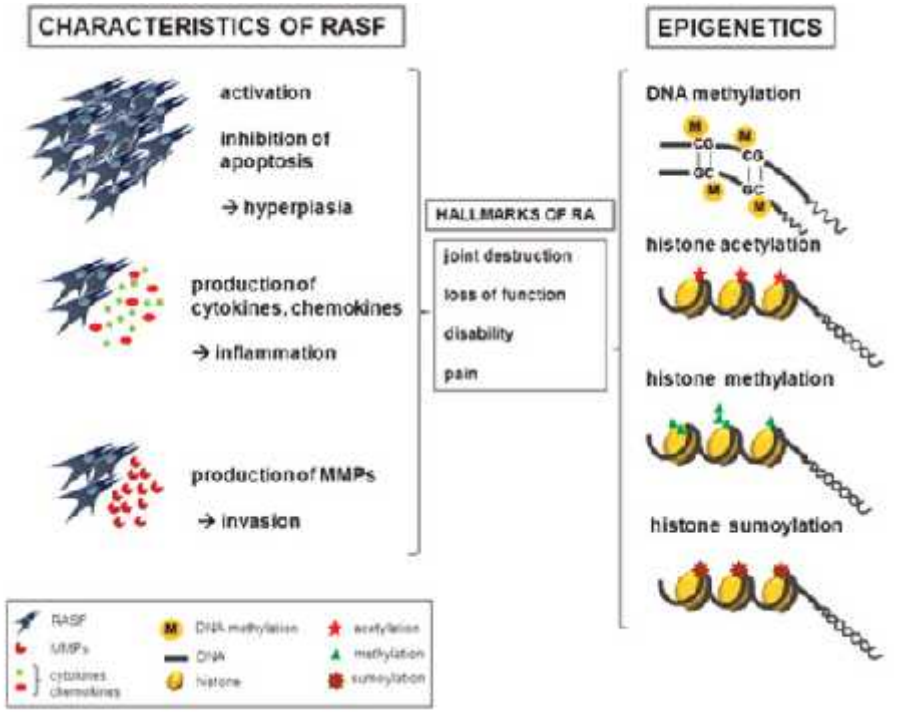
Why chronic inflammation persist?

Epigenetic transformation of RASFs

Cell type	Epigenetic mark	Genomic locus
Monocytes	DNA methylation	Global hypomethylation
B cells	DNA methylation	+/− 2000 DMR: *BARX2, ASB1, ADAMTS12, MGMT* DNA hypomethylation
T cells	DNA methylation	Global hypomethylation; +/− 2000 DMR: *GALNT9, MGMT, CD40L* DNA hypomethylation: *ARSB, DUSP22* DNA hypermethylation
Treg cells	DNA methylation	*CTLA-4* promoter hypermethylation
PBMCs	DNA methylation	> 50'000 DMR: *IL-6* promoter CpG hypomethylation, *IL-10* promoter CpG hypomethylation
Synovial fibroblasts	DNA methylation	Global hypomethylation; +/− 2000 DMR: *CAPN2, EGR, CXCL12, TBX5* DNA hypomethylation, *DPP4, HOXC4* DNA hypermethylation
Synovial fibroblasts	H3K4me3	Increased at the promoters of *MMP-1, MMP-3, MMP-9*, and *MMP-13*
Synovial fibroblasts	H3K27me3	Decreased at the promoters of *MMP-1* and *MMP-9*
Synovial fibroblasts	Histone acetylation	Increased at *MMP-1* promoter and *IL-6* promoter

DMR differentially methylated regions

Semin Immunopathol 2017



Arthritis Research and Therapy 2012

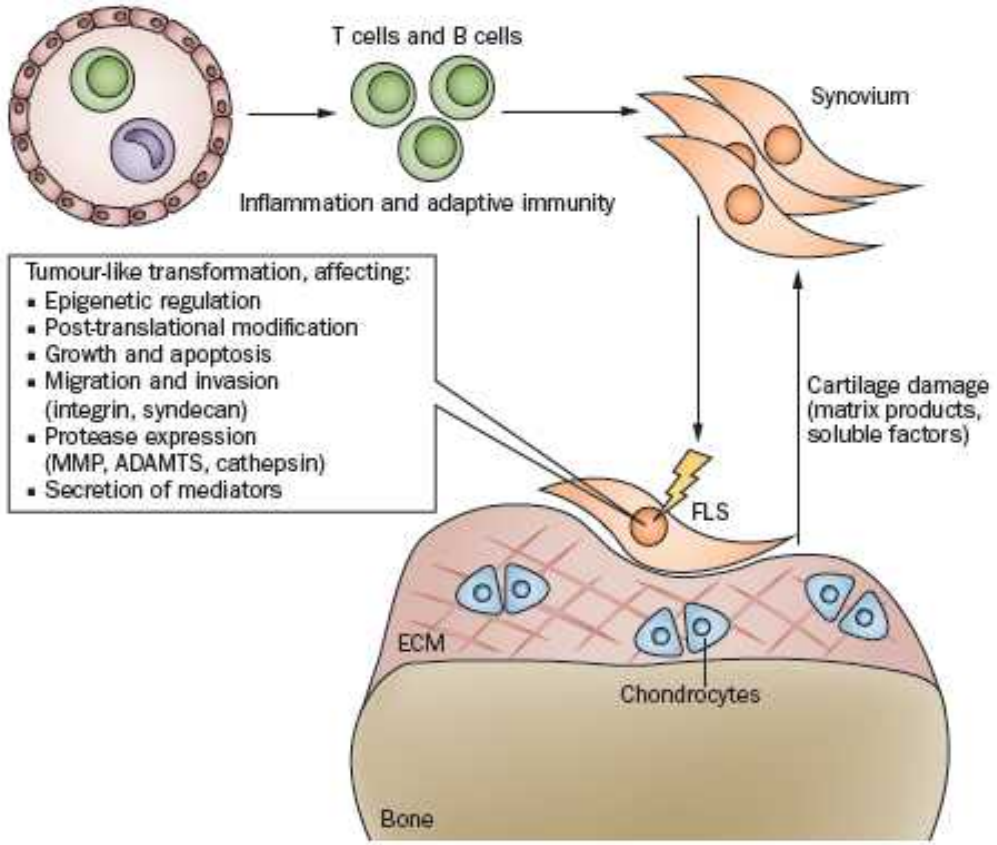
Why chronic inflammation persist?

Permanent transformation of RASFs?

- Apoptosis-resistant

(related to changes in Bcl2, NFkB, PUMA etc)

- Somatic p53 mutations
- Increased migration and invasion
- ECM-related activation (eg tenascinC)



Nature Reviews Rheumatology 2015

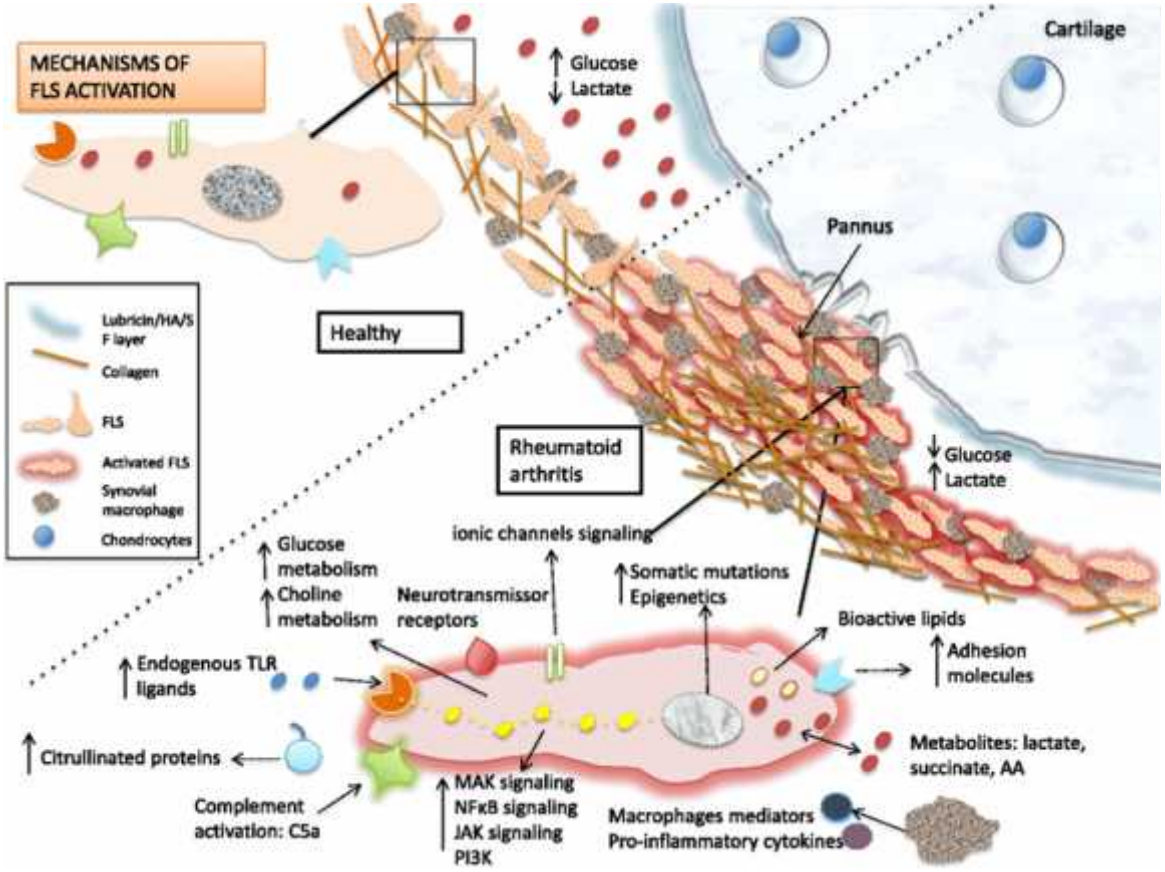
Why chronic inflammation persist?

Augmented glucose metabolism

GLUT1 and other related to glycolytic pathway genes (PKM2, HK2, LDHA and PDK1) are increased in RASFs

GLUT1 augments MMP3 production and cell migration in OA and RA SFs

Hexokinase2 (glucose metabolism enzyme) regulates invasiveness of SFs in mouse models of RA



Arthritis Research and Therapy 2017

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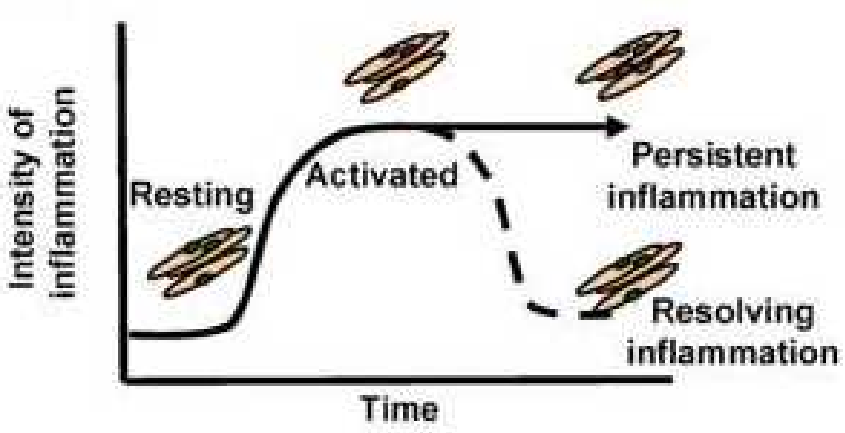
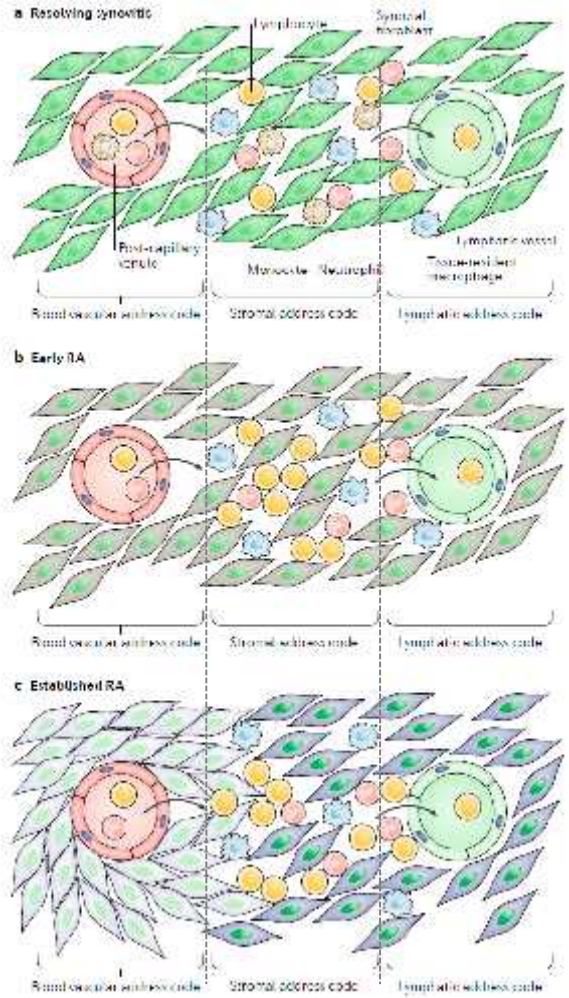
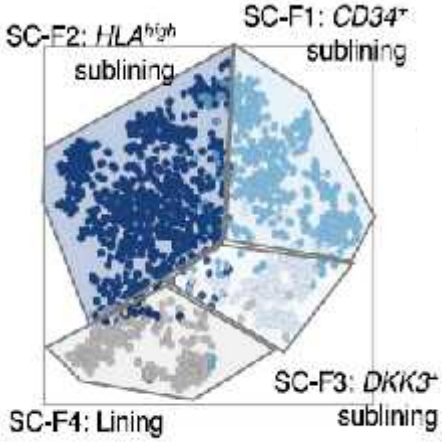




Nature Reviews Rheumatology 2018

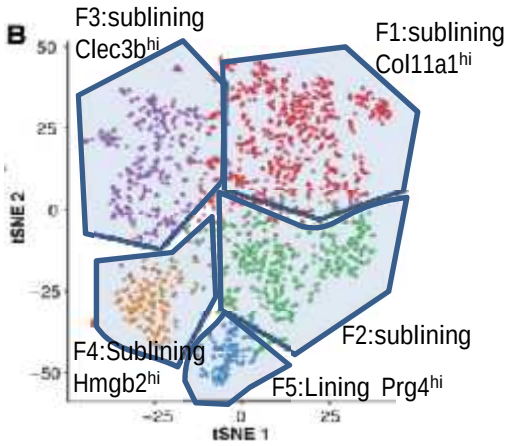
SC transcriptomics: mesenchymal subpopulations identified in RA

Human samples

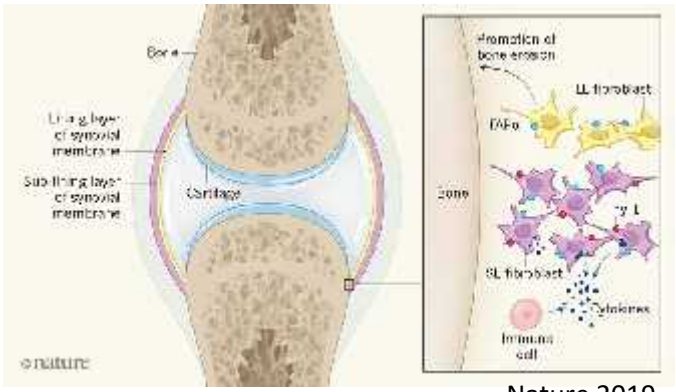


Nature Immunology 2019

Mouse samples



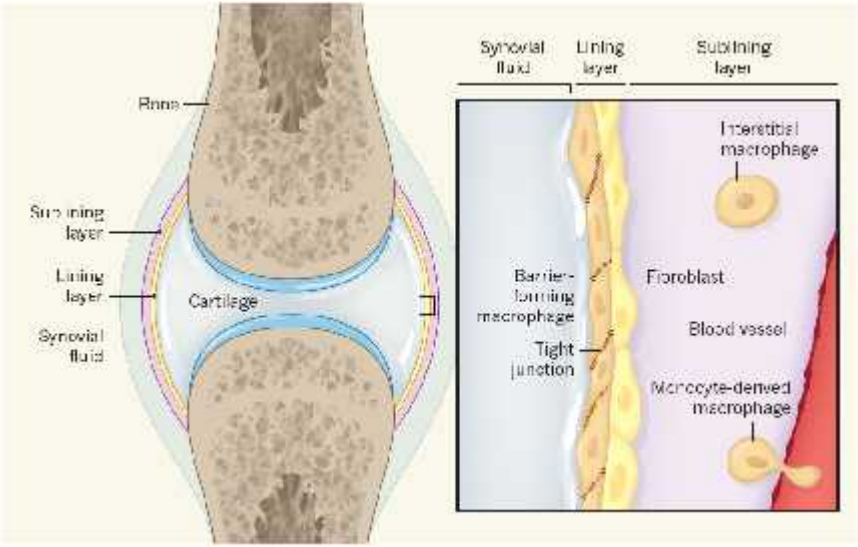
Nature 2019



Nature 2019

SC transcriptomics:
resident and infiltrating myeloid subpopulations identified in health and disease

Is there a role for any of the SF subpopulations in the maintenance of the newly identified synovial barrier-like structure?

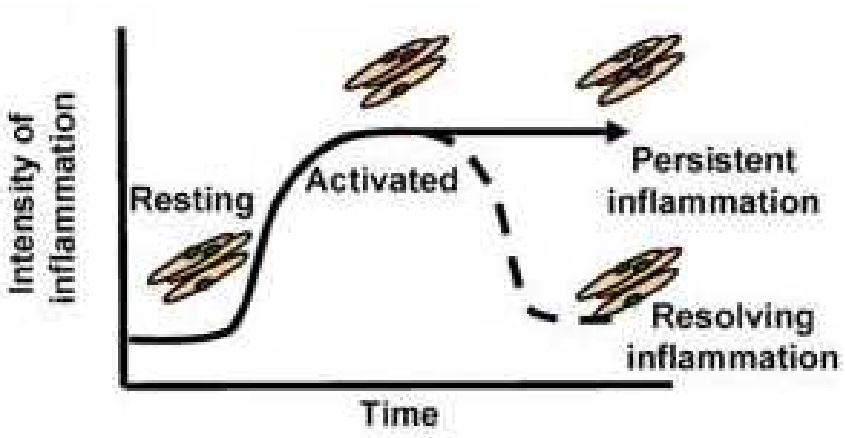


Nature 2019

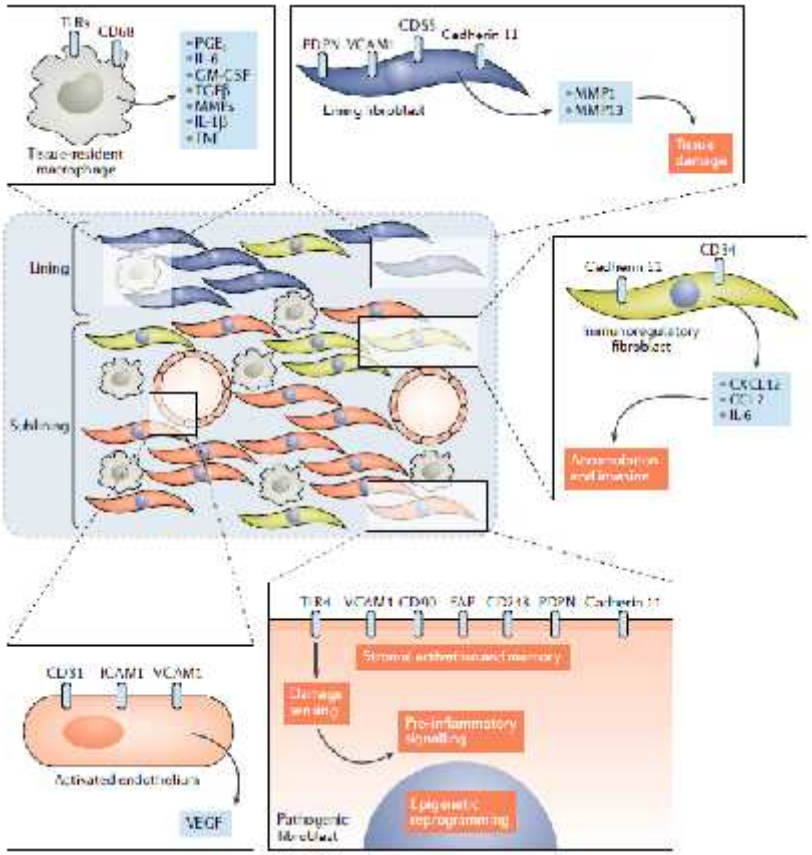
Barrier-forming resident synovial CX_3CR1+ MØs <u>contribute</u> to the protection of synovial membrane by inflammatory attacks

Why chronic inflammation persist?

RA pathogenetic paradigm suggests
a mesenchymal-based hypothesis



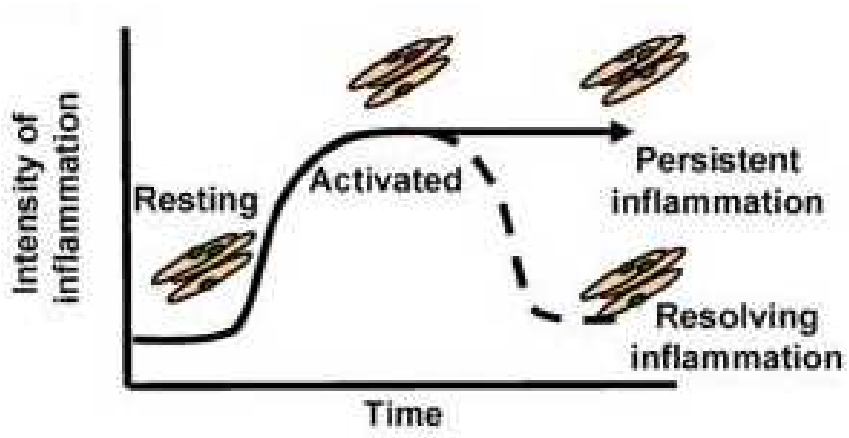
Differential contribution and transformation of synovial subpopulations?



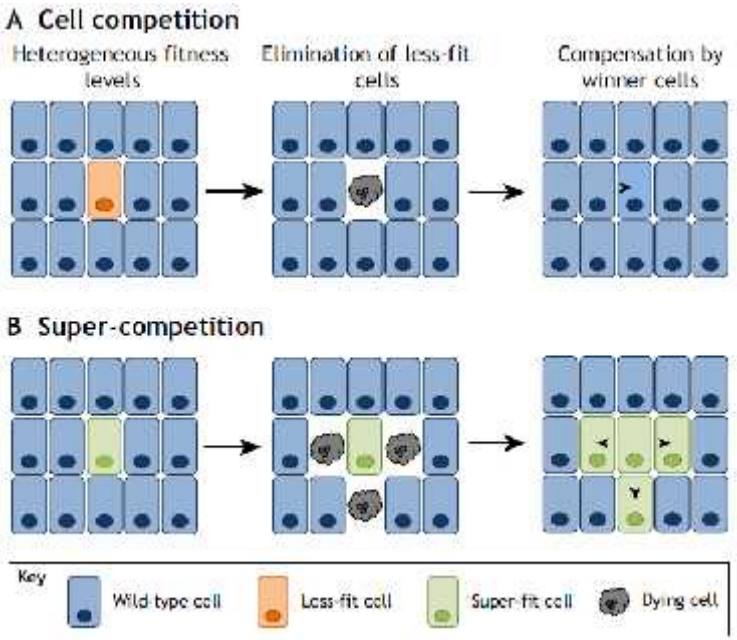
Nature Reviews Rheumatology 2019

Why chronic inflammation persist?

RA pathogenetic paradigm suggests
a mesenchymal-based hypothesis



Differential contribution and transformation of SF subpopulations?

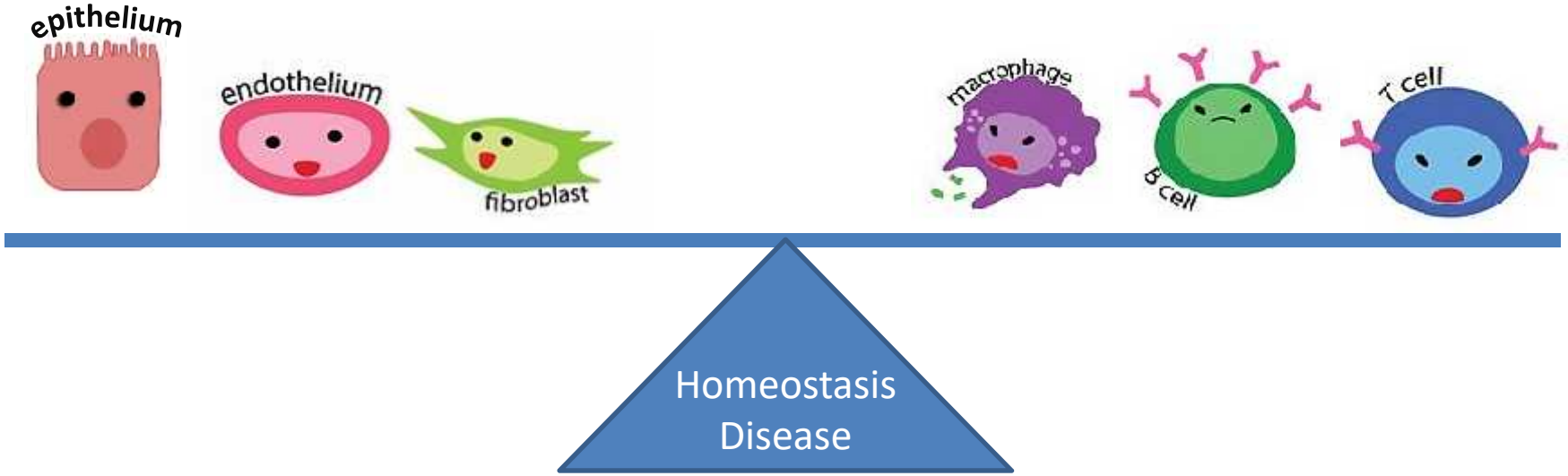


Development 2019

a (mesenchymal) cell competition hypothesis for RA?

Take home message

Stromal cell contribution is equally important in homeostatic and pathogenic immune responses



Take home message

Reciprocal and complementary relationships maintain tissue integrity